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File 155:MEDLINE(R) 1966-1996/Nov W4

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*File 155: Type HELP NEWS 155 for 1996 reload information

*** MEDLINE updates delayed. See HELP DELAY 155.

File 55:BIOSIS PREVIEWS(R) 1985-1996/Oct W2

(c) 1996 BIOSIS

File 73:EMBASE 1974-1996/Iss 40

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Set	Items	Description
S1	27	AU="RING D B"
S2	20	AU="RING DB"
S3	18	AU="RING D.B."
S4	92	AU="RING CS" OR AU="RING D" OR AU="RING D B"
S5	78	RD (unique items)
S6	65	S1 OR S2 OR S3
S7	31	RD (unique items)
S8	2	*BISPECIFIC ANTIBODY 2B 1*
S9	1021	BISPECIFIC(W)ANTIBOD?
S10	5536	CD16
S11	2178	FC(W)GAMMA(W)RECEPTOR?
S12	18042	FC(W)RECEPTOR?
S13	278	*ONCOGENE C ERB*:ONCOGENE C ERBB 2*
S14	173	*ONCOGENE NEU*
S15	4879	CERBB2 OR C(W)ERB(W)B? ? OR C(W)ERBB? ? OR CERBB? ? OR CER- B(W)B? ?
S16	5099	S13 OR S14 OR S15
S17	48	S9 AND S10
S18	8	S17 AND S16
S19	12	S7 AND S9
S20	43	S9 AND S11
S21	100	S9 AND S12
S22	93	S17:S20
S23	59	RD (unique items)
S24	282	ANTIBOD? AND (452F2 OR 741F8 OR 520C9 OR 759E3 OR 113F1 OR 2B1 OR CRL(W)10197 OR HB(W)(10807 OR 10811 OR 8696 OR 10808))
S25	127	RD (unique items)
S26	2247	HER(W)2 OR P185
S27	682	HER2
S28	6879	S16 OR S26 OR S27
S29	61	S24 AND S28

* WELCOME TO THE *
* U.S. PATENT TEXT FILE *

L1	437	NEU OR CERBB2 OR C ERB B# OR C ERBB 2 OR HER2 OR HER 2 OR H
L2	52	BISPECIFIC ANTIBOD? (BISPECIFIC(W)ANTIBOD?)
L3	64	CD16
L4	3	L2 AND L3
L5	0	L1 AND L4
L6	0	L1 AND L2
L7	10	*RING, DAVID B*/IN

?Es19/3,ab/all

19/3,AB/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09700678 96222278

Human neutrophil interactions of a bispecific monoclonal antibody
targeting tumor and human Fc gamma RIIL.

Weiner LM; Alpaugh RK; Amoroso AR; Adams GP; **Ring DB**; Barth MW
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA
19111, USA.lm weiner@fccc.edu

Cancer Immunol Immunother (GERMANY) Mar 1996, 42 (3) p141-50, ISSN
0340-7004 Journal Code: CN3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

2B1 is a bispecific murine monoclonal antibody (bsmAb) targeting the c-erbB-2 and CD16 (Fc gamma RIII) antigens. c-erbB-2 is over-expressed by a variety of adenocarcinomas, and CD16, the low-affinity Fc gamma receptor for aggregated immunoglobulins, is expressed by polymorphonuclear leukocytes (PMN), natural killer (NK) cells and differentiated mononuclear phagocytes. 2B1 potentiates the in vitro lysis of c-erbB-2 over-expressing tumors by NK cells and macrophages. In this report, the interactions between 2B1 and PMN were investigated to assess the impact of these associations on in vitro 2B1-promoted tumor cytotoxicity by human NK cells. The peak binding of 2B1 to PMN was observed at a concentration of 10 microgram/ml 2B1. However, 2B1 rapidly dissociated from PMN in vitro at 37 degrees C in non-equilibrium conditions. This dissociation was not caused by CD16 shedding. When PMN were labeled with 125I-2B1 and incubated at 37 degrees C and the supernatants examined by HPLC analysis, the Fab regions of dissociated 2B1 were not complexed with shed CD16 extracellular domain. While most of the binding of 2B1 PMN was solely attributable to Fab-directed binding to Fc gamma RIII, PMN-associated 2B1 also bound through Fc gamma-domain/Fc gamma RII interactions. 2B1 did not promote in vitro PMN cytotoxicity against c-erbB-2-expressing SK-OV-3 tumor cells. When PMN were coincubated with peripheral blood lymphocytes, SK-OV-3 tumor and 2B1, the concentration of 2B1 required for maximal tumor lysis was lowered. Although PMN may serve as a significant competitive binding pool of systemically administered 2B1 in vivo, the therapeutic potential of the targeted cytotoxicity properties of this bsmAb should not be compromised.

19/3,AB/2 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09673562 96195162

Murine ****bispecific**** ****antibody**** 1A10 directed to human transferrin receptor and a 42-kDa tumor-associated glycoprotein.

Shi T; Hsieh-Ma ST; Reeder J; ****Ring DB****

Department of Immunotherapeutics, Chiron Corporation, Emeryville, California 94608, USA.

Clin Immunol Immunopathol (UNITED STATES) Feb 1996, 78 (2) p188-95, ISSN 0090-1229 Journal Code: DEA

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Previously, we observed that ****bispecific**** ****antibodies**** ("antigen forks") that bound to certain pairs of different tumor surface antigens could inhibit cell growth. The chemically linked heteroconjugate of MAb 454A12 (murine IgG1 recognizing human transferrin receptor) and 317G5 (murine IgG1 recognizing a 42-kDa tumor-associated glycoprotein) was particularly inhibitory toward human colorectal cancer cell lines, and the iron-chelating agent deferoxamine was found to augment inhibition of tumor cell growth by this antigen fork. Further experiments revealed that an antigen fork constructed by linking Fab' fragments instead of whole antibodies retained activity, which led us to construct a fork-secreting hybrid hybridoma. Hybridoma 454A12 was fused with hybridoma 34F2 (murine IgG1 with the same specificity as 317G5). Hybrid hybridomas whose supernatants blocked binding of both 454A12 and 34F2 probes were further tested for the ability to block growth of SW948 human colorectal cancer cells in an MTT growth assay, and were chosen for subcloning. Ascites produced by clone 1A10 was purified by affinity and cation exchange chromatography. Purified 1A10 ****bispecific**** ****antibody**** showed growth inhibitory activity comparable to that of a chemically linked heteroconjugate of its parental antibodies 34F2 and 454A12. Adding deferoxamine greatly enhanced the inhibitory activity of 1A10 and effectively prevented regrowth of tumor cells in vitro. By heterologously crosslinking two antigens that are coexpressed on many tumor cells, this ****bispecific**** ****antibody**** is able to inhibit tumor growth with enhanced selectivity.

19/3,AB/3 (Item 3 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09607882 96129482

Clinical development of 2B1, a bispecific murine monoclonal antibody targeting c-erbB-2 and Fc gamma RIII.

Weiner LM; Clark JI; **Ring DB**; Alpaugh RK

Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA.

J Hematother (UNITED STATES) Oct 1995, 4 (5) p453-6, ISSN 1061-6128

Journal Code: B3T

Contract/Grant No.: CM27732-49, CM, NCI; CA50633, CA, NCI; CA58262, CA, NCI; +

Languages: ENGLISH

Document type: CLINICAL TRIAL; CLINICAL TRIAL, PHASE I; JOURNAL ARTICLE

Bispecific monoclonal antibodies (BsmAb) can be used to specifically target tumor cells for cytotoxicity mediated by defined effector cells. One such BsmAb, 2B1, targets the extracellular domains of both the c-erbB-2 protein product of the HER-2/neu oncogene and Fc gamma RIII (CD16), the Fc gamma receptor expressed by human natural killer cells, neutrophils, and differentiated mononuclear phagocytes. 2B1 promotes the conjugation of cells expressing these target antigens. It efficiently promotes the specific lysis of tumor cells expressing c-erbB-2 by human NK cells and macrophages over a broad concentration range. 2B1 selectively targets c-erbB-2-positive human tumor xenografts growing in immunodeficient SCID mice. Treatment of such mice with 2B1 plus interleukin 2 (IL-2) inhibits the growth of early, established human tumor xenografts overexpressing c-erbB-2. A phase I clinical trial of 2B1 has been initiated to determine the toxicity profile and maximum tolerated dose (MTD) of this BsmAb and to examine the biodistribution of the antibody and the biologic effects of treatment. Preliminary results of this trial indicate that the dose-limiting toxicity for patients with extensive prior bone marrow-toxic therapy is thrombocytopenia for as yet undetermined reasons. Toxicities of fevers, rigors, and associated constitutional symptoms are explained, in part, by treatment-induced systemic expression of cytokines, such as tumor necrosis factor-alpha. Circulating, functional BsmAb is easily detectable in treatment patients' sera and exhibits complex elimination patterns. HAMA and anti-idiotypic treatment-induced antibodies are induced by 2B1 treatment. Some preliminary indications of clinical activity have been observed. BsmAb therapy targeting tumor antigens and Fc gamma RIII has potent immunologic effects. Future studies will include the development of more relevant animal models for BsmAb therapy targeting human Fc gamma RIII. The ongoing phase I trial will be completed to identify the MTD for patients without extensive prior bone marrow-toxic chemotherapy and radiation. A phase II clinical trial of 2B1 therapy in women with metastatic breast cancer is planned, as is a phase I trial incorporating treatment with both 2B1 and IL-2.

19/3,AB/4 (Item 4 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09603286 96124886

Binding characteristics and antitumor properties of 1A10 **bispecific**

antibody recognizing gp40 and human transferrin receptor.

Amoroso AR; Clark JI; Litwin S; Hsieh-Ma S; Shi T; Alpaugh RK; Adams GP;

Wolf EJ; **Ring DB**; Weiner LM

Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA.

Cancer Res (UNITED STATES) Jan 1 1996, 56 (1) p113-20, ISSN 0008-5472

Journal Code: CNF

Contract/Grant No.: CA50633, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The bispecific murine monoclonal antibody (MAb) 1A10 has specificity for the human transferrin receptor (TfR) and the human tumor-associated antigen gp40. This antibody, therefore, functions as an "antigen fork" by binding to two distinct antigens on the same malignant cell. Highly purified 1A10 inhibits the growth of cells coexpressing high levels of human TfR and the tumor-associated antigen gp40 by binding to both target antigens. In SW948 cells, the majority of 1A10 binding is via its gp40 specificity, and half-maximal inhibition of cell growth by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay requires 20-30-micrograms/ml concentrations of 1A10. The binding of 1A10 correlates with growth inhibition in the cell lines HT-29, SK-OV-3, OVCAR-2, and

OVCAR-3. The growth of OVCAR-10 cells, which express little gp40 and TfR, is not inhibited by 1A10. However, SK-BR-3 cells, which express abundant gp40 and extremely high levels of TfR, are insensitive to the effects of 1A10. In some cell lines, combined exposure to 1A10 and the iron chelator deferoxamine mesylate has synergistic antiproliferative effects. A single i.p. dose of 600 micrograms 1A10 is sufficient to achieve an estimated tumor concentration of at least 30 micrograms/ml for 7 days in C.B17/lcr-scld mice bearing SW948 human tumor xenografts. Treatment of scld mice bearing day 2 or day 4 SW948 xenografts with single or multiple 1A10 doses inhibits tumor growth in a dose-related fashion. Antitumor effects are not seen with therapy using either parental antibody of 1A10. The antiproliferative properties of 1A10 in tumor cells overexpressing gp40 and TfR suggest avenues for the development of new **bispecific** **antibody**-promoted treatment strategies.

19/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09484605 96006205

Phase I trial of 2B1, a bispecific monoclonal antibody targeting c-erbB-2 and Fc gamma RIII.

Weiner LM; Clark JI; Davey M; Li WS; Garcia de Palazzo I; **Ring DB**;
Alpaugh RK

Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA.

Cancer Res (UNITED STATES) Oct 15 1995, 55 (20) p4586-93, ISSN 0008-5472 Journal Code: CNF

Contract/Grant No.: 1 RO1 CA50633, CA, NCI; CM-27732-49, CM, NCI; 1 RO1 CA58262, CA, NCI

Languages: ENGLISH

Document type: CLINICAL TRIAL; CLINICAL TRIAL, PHASE I; JOURNAL ARTICLE

2B1 is a bispecific murine monoclonal antibody (BsMAB) with specificity for the c-erbB-2 and Fc gamma RIII extracellular domains. This BsMAB promotes the targeted lysis of malignant cells overexpressing the c-erbB-2 gene product of the HER2/neu proto-oncogene by human natural killer cells and mononuclear phagocytes expressing the Fc gamma RIII A isoform. In a Phase I clinical trial of 2B1, 15 patients with c-erbB-2-overexpressing tumors were treated with 1 h i.v. infusions of 2B1 on days 1, 4, 5, 6, 7, and 8 of a single course of treatment. Three patients were treated with daily doses of 1.0 mg/m², while six patients each were treated with 2.5 mg/m² and 5.0 mg/m², respectively. The principal non-dose-limiting transient toxicities were fevers, rigors, nausea, vomiting, and leukopenia. Thrombocytopenia was dose limiting at the 5.0 mg/m² dose level in two patients who had received extensive prior myelosuppressive chemotherapy. Murine antibody was detectable in serum following 2B1 administration, and its bispecific binding properties were retained. The pharmacokinetics of this murine antibody were variable and best described by nonlinear kinetics with an average t 1/2 of 20 h. Murine antibody bound extensively to all neutrophils and to a proportion of monocytes and lymphocytes. The initial 2B1 treatment induced more than 100-fold increases in circulating levels of tumor necrosis factor-alpha, interleukin 6, and interleukin 8 and lesser rises in granulocyte-monocyte colony-stimulating factor and IFN-gamma. Brisk human anti-mouse antibody responses were induced in 14 of 15 patients. Several minor clinical responses were observed, with reductions in the thickness of chest wall disease in one patient with disseminated breast cancer. Resolution of pleural effusions and ascites, respectively, were noted in two patients with metastatic colon cancer, and one of two liver metastases resolved in a patient with metastatic colon cancer. Treatment with 2B1 BsMAB has potent immunological consequences. The maximum tolerated dose and Phase II daily dose for patients with extensive prior myelosuppressive chemotherapy was 2.5 mg/m². Continued dose escalation is required to identify the maximally tolerated dose for patients who have been less heavily pretreated.

19/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09005116 94320116

Antigen forks: bispecific reagents that inhibit cell growth by binding selected pairs of tumor antigens.

****Ring DB****; Hsieh-Ma ST; Shi T; Reeder J

Department of Immunotherapeutics, Chiron Corporation, Emeryville, CA 94608.

Cancer Immunol Immunother (GERMANY) Jul 1994, 39 (1) p41-8, ISSN 0340-7004 Journal Code: CN3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

****Bispecific**** ****antibodies**** of a new category, termed "antigen forks", were constructed by crosslinking antibodies that recognized pairs of distinct tumor cell surface antigens. At concentrations of 1-100 nM, several such forks inhibited the growth of human tumor cell lines bearing both relevant antigens. The same cells were not inhibited by unconjugated component antibodies, and the active conjugates did not inhibit the growth of human cell lines that expressed lower levels of relevant antigens. The three most active antigen forks all contained monoclonal antibody 454A12, which recognizes human transferrin receptor. This antibody was conjugated respectively to antibodies 113F1 (against a tumor-associated glycoprotein complex), 317G5 (against a 42-kDa tumor-associated glycoprotein), or 520C9 (against the c-erbB-2 protooncogene product). The 317G5-454A12 fork strongly inhibited the HT-29 and SW948 human colorectal cancer cell lines, while the 113F1-454A12 and 520C9-454A12 forks strongly inhibited the SK-BR-3 human breast cancer cell line and the 113F1-454A12 fork was also effective against SW948. By designing forks against antigens of incompatible function that are co-expressed at high levels on tumor cells but not on normal tissues, it may be possible to generate reagents that inhibit tumor growth with enhanced selectivity.

19/3,AB/7 (Item 7 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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08382128 93092128

In vitro cytotoxic targeting by human mononuclear cells and ****bispecific**** ****antibody**** 2B1, recognizing c-erbB-2 protooncogene product and Fc gamma receptor III.

Hsieh-Ma ST; Eaton AM; Shi T; ****Ring DB****

Chiron Corporation, Emeryville, California 94608.

Cancer Res (UNITED STATES) Dec 15 1992, 52 (24) p6832-9, ISSN 0008-5472 Journal Code: CNF

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Bispecific murine monoclonal antibody 2B1, possessing dual specificity for the human c-erbB-2 protooncogene product and human Fc gamma receptor III (CD16) was evaluated for the ability to promote specific lysis of c-erbB-2-positive tumor cells in vitro. In short-term ⁵¹Cr release assays with human mononuclear cells as effectors and SK-BR-3 human breast cancer cells as targets, neither parental antibody of 2B1 mediated significant specific lysis, but ****bispecific**** ****antibody**** was as active as a chemical heteroconjugate, with 5 ng/ml of 2B1 causing half-maximal lysis at an effector/target ratio of 20:1 and 2 ng/ml 2B1 causing half-maximal lysis at an E/T ratio of 40:1. The cytotoxic targeting activity of 2B1 F(ab')₂ fragment was the same as that of whole ****bispecific**** ****antibody****, and the activity of whole 2B1 was not reduced when assays were performed in 100% autologous human serum, indicating that 2B1 binds effector cells through the CD16-binding site derived from parental antibody 3G8 rather than through its Fc portion. Variable inhibition of 2B1-mediated lysis was observed when autologous polymorphonuclear leukocytes from different donors were added to mononuclear effector cells at a 2:1 ratio; this inhibition was overcome at higher antibody concentration. 2B1 bispecific monoclonal antibody was also able to mediate targeted cytolysis using whole human blood as a source of effector cells or using effector or target cells derived from ovarian cancer patients.

19/3,AB/8 (Item 8 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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07830601 91349601

Selection of hybrid hybridomas by flow cytometry using a new combination of fluorescent vital stains.

Shi T; Eaton AM; **Ring DB**

Department of Immunology, Cetus Corporation, Emeryville, CA 94608.

J Immunol Methods (NETHERLANDS) Aug 9 1991, 141 (2) p165-75, ISSN 0022-1759 Journal Code: IFE

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A new combination of fluorescent dyes (rhodamine 123 and hydroethidine) was used to internally label hybridoma fusion partners. Murine hybridoma 520C9 (recognizing human c-erbB-2) was labeled with hydroethidine. Murine hybridoma 3G8 (recognizing human Fc gamma receptor III) was labeled with rhodamine 123, and verapamil was used to block rhodamine efflux via P-glycoprotein. Viability assays showed little cytotoxicity from these dyes at the concentrations used. The labeled cells were fused with polyethylene glycol, sorted for dual fluorescence on an Epics V cell sorter, and cloned. Hybrid hybridomas producing **bispecific** **antibodies** were selected for ability to promote lysis of SK-Br-3 breast cancer cells by human mononuclear cells. Several positive clones were obtained and shown to have a double content of DNA. **Bispecific** **antibody** produced by subclone 2B1 was purified by anion exchange chromatography and shown to bind both tumor cells and Fc gamma R III bearing cells. Using two parameter flow cytometric analysis, we were able to measure a 'bridging' effect of this **bispecific** **antibody**, which caused formation of complexes between PMNs and SK-Br-3 cells. Either parental antibody could compete with **bispecific** **antibody** to block such complexing. This fusion method provides several advantages over other techniques presently used (speed, convenience, low toxicity and automatic exclusion of dead cells) and can be applied to produce other hybrid hybridomas.

19/3,AB/9 (Item 1 from file: 55)

DIALOG(R)File 55:BIOSIS PREVIEWS(R)

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13138282 BIOSIS Number: 99138282

In vitro tumor growth inhibition by **bispecific** **antibodies** to human transferrin receptor and tumor-associated antigens is augmented by the iron chelator deferoxamine

Hsieh-Ma S T; Shi T; Reeder J; **Ring D B**

Dep. Immunotherapeutics M400, Chiron Corp., 4560 Horton St., Emeryville, CA 94608, USA

Clinical Immunology and Immunopathology 80 (2). 1996. 185-193.

Full Journal Title: Clinical Immunology and Immunopathology

ISSN: 0090-1229

Language: ENGLISH

Print Number: Biological Abstracts Vol. 102 Iss. 006 Ref. 086413

Previously, a panel of mouse monoclonal antibodies (mAbs) to several tumor-associated antigens was chemically crosslinked to an IgG1 anti-human transferrin receptor antibody, 454A12. We called this new class of **bispecific** **antibodies** (BmAbs) "antigen forks" and showed that these antigen forks inhibited but did not completely prevent tumor cell growth. We speculated that the conjugates acted by heterologously crosslinking two antigens in a manner that interfered with the functions of one or both. The most effective BmAbs all shared one specificity for the human transferrin receptor. A monoclonal antibody to this receptor has been shown by others to reduce tumor cell growth when used with the iron chelator deferoxamine. When we combined our antigen forks with deferoxamine, two of five BmAbs synergized with deferoxamine to arrest tumor cell count at or below input levels. The most effective BmAbs were 317G5/454A12 (3/4) and 520C9/454A12 (5/4). mAb 317G5 recognizes a 42-kDa tumor-associated glycoprotein, and mAb 520C9 recognizes the c-erbB-2 protooncogene product. BmAb 3/4 was most effective against colorectal cancer cell line HT-29, and BmAb 5/4 was most effective against breast cancer cell line SK-BR-3. When deferoxamine and BmAb were replaced by fresh medium after a 6- or 7-day treatment period, no regrowth of tumor cells was observed during the next 4 days, although regrowth was seen if either deferoxamine or BmAb was used alone. Our results show that BmAbs with specificities for transferrin receptor and certain tumor-associated antigens effectively inhibit tumor growth in vitro. When used in combination with deferoxamine, such BmAbs may have therapeutic potential for the treatment of cancer.

19/3,AB/10 (Item 2 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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12083293 BIOSIS Number: 98683293

Binding characteristics and antitumor properties of 1A10 ****bispecific****
****antibody**** recognizing gp40 and human transferrin receptor
Amoroso A R; Clark J I; Litwin S; Hsieh-Ma S; Shi T; Alpaugh R K; Adams G
P; Wolff E J; ****Ring D B****; Weiner L M
Dep. Med. Oncol., Fox Chase Cancer Cent., 7701 Burholme Ave.,
Philadelphia, PA 19111, USA
Cancer Research 56 (1). 1996. 113-120.
Full Journal Title: Cancer Research
ISSN: 0008-5472
Language: ENGLISH

Print Number: Biological Abstracts Vol. 101 Iss. 006 Ref. 083574
The bispecific murine monoclonal antibody (MAb) 1A10 has specificity for the human transferrin receptor (TfR) and the human tumor-associated antigen gp40. This antibody, therefore, functions as an "antigen fork" by binding to two distinct antigens on the same malignant cell. Highly purified 1A10 inhibits the growth of cells coexpressing high levels of human TfR and the tumor-associated antigen gp40 by binding to both target antigens. In SW948 cells, the majority of 1A10 binding is via its gp40 specificity, and half-maximal inhibition of cell growth by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay requires 2030-mu-g/ml concentrations of 1A10. The binding of 1A10 correlates with growth inhibition in the cell lines HT-29, SK-OV-3, OVCAR-2, and OVCAR-3. The growth of OVCAR-10 cells, which express little gp40 and TfR, is not inhibited by 1A10. However, SK-BR-3 cells, which express abundant gp40 and extremely high levels of TfR, are insensitive to the effects of 1A10. In some cell lines, combined exposure to 1A10 and the iron chelator deferoxamine mesylate has synergistic antiproliferative effects. A single i.p. dose of 600 mu-g 1A10 is sufficient to achieve an estimated tumor concentration of at least 30 mu-g/ml for 7 days in C.B17/lcr-scid mice bearing SW948 human tumor xenografts. Treatment of scid mice bearing day 2 or day 4 SW948 xenografts with single or multiple 1A10 doses inhibits tumor growth in a dose-related fashion. Antitumor effects are not seen with therapy using either parental antibody of 1A10. The antiproliferative properties of 1A10 in tumor cells overexpressing gp40 and TfR suggest avenues for the development of new ****bispecific**** ****antibody****-promoted treatment strategies.

19/3,AB/11 (Item 3 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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11138919 BIOSIS Number: 97338919

Improvements in hybridoma culture and ****bispecific**** ****antibody**** production
Inlow D; Lowe D; Howarth B; MacDonald H; Harano D; Davis J; Maiorella B; Brannon M; Fordham D; Lin L; Reeder J; ****Ring D B****
Chiron Corp., Emeryville, CA 94608, USA
Journal of Cellular Biochemistry Supplement 0 (18D). 1994. 188.
Full Journal Title: Keystone Symposium on Antibody Engineering: Research and Application of Genes Encoding Immunoglobulins, Lake Tahoe, California, USA, March 7-13, 1994. Journal of Cellular Biochemistry Supplement
ISSN: 0733-1959
Language: ENGLISH
Document Type: CONFERENCE PAPER
Print Number: Biological Abstracts/RRM Vol. 046 Iss. 008 Ref. 116637

19/3,AB/12 (Item 4 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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7328223 BIOSIS Number: 38108744

HYBRID HYBRIDOMA PRODUCING ****BISPECIFIC**** ****ANTIBODIES**** TO HUMAN BREAST CANCER ASSOCIATED ANTIGENS AND HUMAN FC RECEPTOR III
SHI T; ****RING D B****; EATON A M; HSIEH-MA S T; KASSEL J A

DEP. IMMUNOL., CUTS CORP., EMERYVILLE, CALIF.
XIVTH INTERNATIONAL MEETING OF THE SOCIETY FOR ANALYTICAL CYTOLOGY,
ASHEVILLE, NORTH CAROLINA, USA, MARCH 18-23, 1990. CYTOMETRY 0 (SUPPL. 4).
1990. 72. CODEN: CYTOD
Language: ENGLISH
Document Type: CONFERENCE PAPER
?Ds8/3,ab/all

8/3,AB/1 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
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9594349 EMBASE No: 95154296
Bispecific antibodies and targeted cellular cytotoxicity
Drakeman D.L.; Fanger M.W.
Medarex, Inc., 1545 Route 22 East, Annandale, NJ 08801-0953 USA
Drug News and Perspectives (Spain) , 1995, 8/3 (189-192) CODEN: DNPEE
ISSN: 0214-0934
LANGUAGES: English

8/3,AB/2 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE
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9594341 EMBASE No: 95154288
Bispecific antibodies
Fanger M.W.; Drakeman D.L.
Dartmouth Medical School, Lebanon, NH USA
Drug News and Perspectives (Spain) , 1995, 8/3 (133-137) CODEN: DNPEE
ISSN: 0214-0934
LANGUAGES: English

?Ds23/3,ab/all

23/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09700678 96222278
Human neutrophil interactions of a bispecific monoclonal antibody
targeting tumor and human Fc gamma RIIL.
Weiner LM; Alpaugh RK; Amoroso AR; Adams GP; **Ring DB**; Barth MW
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA
19111, USA.lm weiner@fccc.edu
Cancer Immunol Immunother (GERMANY) Mar 1996, 42 (3) p141-50, ISSN
0340-7004 Journal Code: CN3
Languages: ENGLISH
Document type: JOURNAL ARTICLE
2B1 is a bispecific murine monoclonal antibody (bsmAb) targeting the
c-**erbB**-2 and **CD16** (Fc gamma RIIL) antigens. **c**-**erbB**-2 is
over-expressed by a variety of adenocarcinomas, and **CD16**, the
low-affinity **Fc** **gamma** **receptor** for aggregated immunoglobulins,
is expressed by polymorphonuclear leukocytes (PMN), natural killer (NK)
cells and differentiated mononuclear phagocytes. 2B1 potentiates the in
vitro lysis of c-erb-2 over-expressing tumors by NK cells and macrophages.
In this report, the interactions between 2B1 and PMN were investigated to
assess the impact of these associations on in vitro 2B1-promoted tumor
cytotoxicity by human NK cells. The peak binding of 2B1 to PMN was observed
at a concentration of 10 microgram/ml 2B1. However, 2B1 rapidly dissociated
from PMN in vitro at 37 degrees C in non-equilibrium conditions. This
dissociation was not caused by **CD16** shedding. When PMN were labeled
with 125I-2B1 and incubated at 37 degrees C and the supernatants examined
by HPLC analysis, the Fab regions of dissociated 2B1 were not complexed
with shed **CD16** extracellular domain. While most of the binding of 2B1
PMN was solely attributable to Fab-directed binding to Fc gamma RIIL,
PMN-associated 2B1 also bound through Fc gamma-domain/Fc gamma RII
interactions. 2B1 did not promote in vitro PMN cytotoxicity against **c**-
erbB-2-expressing SK-OV-3 tumor cells. When PMN were coincubated with
peripheral blood lymphocytes, SK-OV-3 tumor and 2B1, the concentration of

2B1 required for maximal tumor lysis was lowered. Although PMN may serve as a significant competitive binding pool of systemically administered 2B1 in vivo, the therapeutic potential of the targeted cytotoxicity properties of this bsmAb should not be compromised.

23/3,AB/2 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09675765 96197365

Interleukin-12 increases ****bispecific****-****antibody****-mediated natural killer cell cytotoxicity against human tumors.

Sahin U; Kraft-Bauer S; Ohnesorge S; Pfreundschuh M; Renner C
Med. Klinik I, University of Saarland Medical School, Homburg/Saar, Germany.

Cancer Immunol Immunother (GERMANY) Jan 1996, 42 (1) p9-14, ISSN 0340-7004 Journal Code: CN3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The combination of ****CD16****/CD30 bispecific monoclonal antibodies (bi-mAb) and unstimulated human resting natural killer (NK) cells can cure about 50% of mice with severe combined immunodeficiency (SCID) bearing subcutaneously growing established Hodgkin's lymphoma. As interleukin-2 (IL-2) and IL-12 have been shown to increase NK cell activity, we tested the capacity of these cytokines to increase bi-mAb-mediated NK cell cytotoxicity against two types of human tumors (Hodgkin's disease and colorectal carcinoma). Unstimulated NK cells needed a three- to five-times higher antibody concentration than cytokine-stimulated NK cells to exert similar levels of bi-mAb-mediated cytotoxicity. The augmented tumor cell lysis was achieved with IL-12 at considerably lower concentrations than with IL-2 and was associated with a significantly increased bi-mAb-mediated intracellular Ca²⁺ mobilization. The efficiency of IL-12 in this setting together with its low toxicity make it the ideal candidate for a combination therapy with NK-cell-activating bi-mAb in human tumors that are resistant to standard treatment.

23/3,AB/3 (Item 3 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09673562 96195162

Murine ****bispecific**** ****antibody**** 1A10 directed to human transferrin receptor and a 42-kDa tumor-associated glycoprotein.

Shi T; Hsieh-Ma ST; Reeder J; ****Ring DB****

Department of Immunotherapeutics, Chiron Corporation, Emeryville, California 94608, USA.

Clin Immunol Immunopathol (UNITED STATES) Feb 1996, 78 (2) p188-95, ISSN 0090-1229 Journal Code: DEA

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Previously, we observed that ****bispecific**** ****antibodies**** ("antigen forks") that bound to certain pairs of different tumor surface antigens could inhibit cell growth. The chemically linked heteroconjugate of MAb 454A12 (murine IgG1 recognizing human transferrin receptor) and 317G5 (murine IgG1 recognizing a 42-kDa tumor-associated glycoprotein) was particularly inhibitory toward human colorectal cancer cell lines, and the iron-chelating agent deferoxamine was found to augment inhibition of tumor cell growth by this antigen fork. Further experiments revealed that an antigen fork constructed by linking Fab' fragments instead of whole antibodies retained activity, which led us to construct a fork-secreting hybrid hybridoma. Hybridoma 454A12 was fused with hybridoma 34F2 (murine IgG1 with the same specificity as 317G5). Hybrid hybridomas whose supernatants blocked binding of both 454A12 and 34F2 probes were further tested for the ability to block growth of SW948 human colorectal cancer cells in an MTT growth assay, and were chosen for subcloning. Ascites produced by clone 1A10 was purified by affinity and cation exchange chromatography. Purified 1A10 ****bispecific**** ****antibody**** showed growth inhibitory activity comparable to that of a chemically linked heteroconjugate of its parental antibodies 34F2 and 454A12. Adding deferoxamine greatly enhanced the inhibitory activity of 1A10 and

effectively prevented regrowth of tumor cells in vitro. By heterologously crosslinking two antigens that are coexpressed on many tumor cells, this **bispecific antibody** is able to inhibit tumor growth with enhanced selectivity.

23/3,AB/4 (Item 4 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09607883 96129483

Targeting lipoproteins to **Fc gamma receptors** with **bispecific antibodies**.

Morganelli PM

Veterans Administration Hospital, White River Jct., VT, USA.

J Hematother (UNITED STATES) Oct 1995, 4 (5) p457-61, ISSN 1061-6128

Journal Code: B3T

Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL

The data presented here support use of **bispecific antibodies** (BsAb) for studying the role of each of the different types of IgG Fc receptors (Fc gamma Rs) in uptake and metabolism of low-density lipoprotein (LDL) immune complexes. The bispecific anti-Fc gamma R x anti-LDL antibodies used in these studies were effective in specifically triggering metabolic uptake and degradation of LDL immune complexes (LDL-IC) through each type of Fc gamma R. Using LDL-IC prepared with LDL aggregates, foam cell formation was induced with relatively acute stimulation. Thus, these conditions will be appropriate for studying lipoprotein metabolism in the context of specific Fc gamma R under a variety of conditions to determine if, in fact, there are differences in sterol metabolism associated with the different types of Fc gamma R and for comparing metabolism to that mediated by the other important pathways of lipoprotein uptake (native LDL receptors and scavenger receptors). The results of these studies will reveal which of these pathways are potentially most important in foam cell formation and might suggest the possibility that macrophage foam cell formation could be altered by redirecting LDL to particular pathways.

23/3,AB/5 (Item 5 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09607882 96129482

Clinical development of 2B1, a bispecific murine monoclonal antibody targeting **c-erbB-2** and Fc gamma RIII.

Weiner LM; Clark JI; Ring DB*; Alpaugh RK

Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA.

J Hematother (UNITED STATES) Oct 1995, 4 (5) p453-6, ISSN 1061-6128

Journal Code: B3T

Contract/Grant No.: CM27732-49, CM, NCI; CA50633, CA, NCI; CA58262, CA, NCI; +

Languages: ENGLISH

Document type: CLINICAL TRIAL; CLINICAL TRIAL, PHASE I; JOURNAL ARTICLE

Bispecific monoclonal antibodies (BsmAb) can be used to specifically target tumor cells for cytotoxicity mediated by defined effector cells. One such BsmAb, 2B1, targets the extracellular domains of both the **c-erbB-2** protein product of the HER-2/neu oncogene and Fc gamma RIII (**CD16**), the **Fc gamma receptor** expressed by human natural killer cells, neutrophils, and differentiated mononuclear phagocytes. 2B1 promotes the conjugation of cells expressing these target antigens. It efficiently promotes the specific lysis of tumor cells expressing **c-erbB-2** by human NK cells and macrophages over a broad concentration range. 2B1 selectively targets **c-erbB-2**-positive human tumor xenografts growing in immunodeficient SCID mice. Treatment of such mice with 2B1 plus interleukin 2 (IL-2) inhibits the growth of early, established human tumor xenografts overexpressing **c-erbB-2**. A phase I clinical trial of 2B1 has been initiated to determine the toxicity profile and maximum tolerated dose (MTD) of this BsmAb and to examine the biodistribution of the antibody and the biologic effects of treatment. Preliminary results of this trial indicate that the dose-limiting toxicity for patients with extensive prior bone marrow-toxic therapy is

thrombocytopenia for as yet undetermined reasons. Toxicities of fevers, rigors, and associated constitutional symptoms are explained, in part, by treatment-induced systemic expression of cytokines, such as tumor necrosis factor-alpha. Circulating, functional BsmAb is easily detectable in treatment patients' sera and exhibits complex elimination patterns. HAMA and anti-idiotypic treatment-induced antibodies are induced by 2B1 treatment. Some preliminary indications of clinical activity have been observed. BsmAb therapy targeting tumor antigens and Fc gamma RIII has potent immunologic effects. Future studies will include the development of more relevant animal models for BsmAb therapy targeting human Fc gamma RIII. The ongoing phase I trial will be completed to identify the MTD for patients without extensive prior bone marrow-toxic chemotherapy and radiation. A phase II clinical trial of 2B1 therapy in women with metastatic breast cancer is planned, as is a phase I trial incorporating treatment with both 2B1 and IL-2.

23/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09607881 96129481

Treatment of heterotransplanted Hodgkin's tumors in SCID mice by a combination of human NK or T cells and ****bispecific** antibodies****.
Renner C; Pfreundschuh M
Medical Department I, University of the Saarland, Homburg, Germany.
J Hematother (UNITED STATES) Oct 1995, 4 (5) p447-51, ISSN 1061-6128
Journal Code: B3T
Languages: ENGLISH
Document type: JOURNAL ARTICLE

To test the feasibility and efficacy of a new immunotherapeutic approach in Hodgkin's disease, bispecific monoclonal antibodies (BsmAb) were established with specificity for the Hodgkin's-associated CD30 antigen and for ****CD16**** (on NK cells) or CD3 and CD28 (on T lymphocytes), respectively. These BsmAb induced a specific and efficient NK cell or T cell-mediated cytotoxicity in vitro. The treatment of severe combined immunodeficiency (SCID) mice with the NK (anti-****CD16****/CD30) or T cell (anti-CD3/CD30 and anti-CD28/CD30) activating BsmAb followed by administration of resting human lymphocytes led to complete remission of established heterotransplanted human Hodgkin's tumors. Even disseminated tumors were cured. Studies on the mechanism responsible for tumor destruction revealed that treatment efficacy depended on lymphocyte activation at the tumor site. Localization of human lymphocytes in mice was BsmAb mediated and antigen specific as activated lymphocytes were only detected in CD30+ tumors but not in CD30- colorectal carcinomas co-established as a control in the same animal.

23/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09603286 96124886

Binding characteristics and antitumor properties of 1A10 ****bispecific** antibody**** recognizing gp40 and human transferrin receptor.
Amoroso AR; Clark JI; Litwin S; Hsieh-Ma S; Shi T; Alpaugh RK; Adams GP; Wolf EJ; ****Ring DB****; Weiner LM
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA.
Cancer Res (UNITED STATES) Jan 1 1996, 56 (1) p113-20, ISSN 0008-5472
Journal Code: CNF
Contract/Grant No.: CA50633, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The bispecific murine monoclonal antibody (MAb) 1A10 has specificity for the human transferrin receptor (TfR) and the human tumor-associated antigen gp40. This antibody, therefore, functions as an "antigen fork" by binding to two distinct antigens on the same malignant cell. Highly purified 1A10 inhibits the growth of cells coexpressing high levels of human TfR and the tumor-associated antigen gp40 by binding to both target antigens. In SW948 cells, the majority of 1A10 binding is via its gp40 specificity, and half-maximal inhibition of cell growth by 3-(4,

5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay requires 20-30-micrograms/ml concentrations of 1A10. The binding of 1A10 correlates with growth inhibition in the cell lines HT-29, SK-OV-3, OVCAR-2, and OVCAR-3. The growth of OVCAR-10 cells, which express little gp40 and TfR, is not inhibited by 1A10. However, SK-BR-3 cells, which express abundant gp40 and extremely high levels of TfR, are insensitive to the effects of 1A10. In some cell lines, combined exposure to 1A10 and the iron chelator deferoxamine mesylate has synergistic antiproliferative effects. A single i.p. dose of 600 micrograms 1A10 is sufficient to achieve an estimated tumor concentration of at least 30 micrograms/ml for 7 days in C.B17/lcr-scld mice bearing SW948 human tumor xenografts. Treatment of scld mice bearing day 2 or day 4 SW948 xenografts with single or multiple 1A10 doses inhibits tumor growth in a dose-related fashion. Antitumor effects are not seen with therapy using either parental antibody of 1A10. The antiproliferative properties of 1A10 in tumor cells overexpressing gp40 and TfR suggest avenues for the development of new **bispecific** **antibody** **-promoted treatment strategies.**

23/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09484605 96006205

Phase I trial of 2B1, a bispecific monoclonal antibody targeting c-erbB-2 and Fc gamma RIII.

Weiner LM; Clark JI; Davey M; Li WS; Garcia de Palazzo I; **Ring DB**;
Alpaugh RK

Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA.

Cancer Res (UNITED STATES) Oct 15 1995, 55 (20) p4586-93, ISSN 0008-5472 Journal Code: CNF

Contract/Grant No.: 1 RO1 CA50633, CA, NCI; CM-27732-49, CM, NCI; 1 RO1 CA58262, CA, NCI

Languages: ENGLISH

Document type: CLINICAL TRIAL; CLINICAL TRIAL, PHASE I; JOURNAL ARTICLE

2B1 is a bispecific murine monoclonal antibody (BsMab) with specificity for the c-erbB-2 and Fc gamma RIII extracellular domains. This BsMab promotes the targeted lysis of malignant cells overexpressing the c-erbB-2 gene product of the HER2/neu proto-oncogene by human natural killer cells and mononuclear phagocytes expressing the Fc gamma RIII A isoform. In a Phase I clinical trial of 2B1, 15 patients with c-erbB-2-overexpressing tumors were treated with 1 h i.v. infusions of 2B1 on days 1, 4, 5, 6, 7, and 8 of a single course of treatment. Three patients were treated with daily doses of 1.0 mg/m², while six patients each were treated with 2.5 mg/m² and 5.0 mg/m², respectively. The principal non-dose-limiting transient toxicities were fevers, rigors, nausea, vomiting, and leukopenia. Thrombocytopenia was dose limiting at the 5.0 mg/m² dose level in two patients who had received extensive prior myelosuppressive chemotherapy. Murine antibody was detectable in serum following 2B1 administration, and its bispecific binding properties were retained. The pharmacokinetics of this murine antibody were variable and best described by nonlinear kinetics with an average t_{1/2} of 20 h. Murine antibody bound extensively to all neutrophils and to a proportion of monocytes and lymphocytes. The initial 2B1 treatment induced more than 100-fold increases in circulating levels of tumor necrosis factor-alpha, interleukin 6, and interleukin 8 and lesser rises in granulocyte-monocyte colony-stimulating factor and IFN-gamma. Brisk human anti-mouse antibody responses were induced in 14 of 15 patients. Several minor clinical responses were observed, with reductions in the thickness of chest wall disease in one patient with disseminated breast cancer. Resolution of pleural effusions and ascites, respectively, were noted in two patients with metastatic colon cancer, and one of two liver metastases resolved in a patient with metastatic colon cancer. Treatment with 2B1 BsMab has potent immunological consequences. The maximum tolerated dose and Phase II daily dose for patients with extensive prior myelosuppressive chemotherapy was 2.5 mg/m². Continued dose escalation is required to identify the maximally tolerated dose for patients who have been less heavily pretreated.

23/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

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09411188 95341188

Enhanced metabolism of LDL aggregates mediated by specific human monocyte IgG Fc receptors.

Morganelli PM; Rogers RA; Kitzmiller TJ; Bergeron A
Veteran's Administration Hospital, VT 05009, USA.

J Lipid Res (UNITED STATES) Apr 1995, 36 (4) p714-24, ISSN 0022-2275
Journal Code: IX3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Macrophage-derived foam cells are important constituents of atheromatous lesions. In addition to the scavenger receptor pathway, uptake of immune complexed lipoproteins through IgG Fc receptors (**Fc** **gamma** **receptors**) represents an additional pathway of macrophage foam cell development that may be important during atherogenesis. The importance of this mechanism is suggested by studies showing that the titer of autoantibodies to modified lipoproteins correlated with the extent of occlusive disease in patients, and that those antibodies exist in human lesions. Human mononuclear phagocytes possess three structurally and functionally distinct classes of **Fc** **gamma** **receptors**, each of which could be associated with a unique pathway of lipoprotein metabolism. In order to determine whether uptake of an acute lipid load through each type of **Fc** **gamma** **receptor** was associated with foam cell development, we used **bispecific** **antibodies** consisting of anti-LDL monoclonal antibodies conjugated to anti-**Fc** **gamma** **receptor** monoclonal antibodies to study the effects of targeting LDL aggregates to each specific type of **Fc** **gamma** **receptor** on freshly isolated adherent human monocytes. Relative to appropriate controls, LDL degradation, cellular sterol mass, and foam cell development of monocytes were enhanced by targeting LDL aggregates to Fc gamma RI or Fc gamma RII, and this was accompanied by an apparent impairment of LDL degradation. Uptake was specific to the **Fc** **gamma** **receptors** and was not influenced by the presence of scavenger receptor ligands. Thus, with the bispecific approach, the functions of each class of **Fc** **gamma** **receptor** can be studied on an individual basis with respect to several aspects of cellular cholesterol metabolism. This will be critical for determining which of these receptors are potentially most important in the clearance of lipoprotein immune complexes during atherogenesis.

23/3,AB/10 (Item 10 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09360386 95290386

Potentiation of lysis of leukaemia cells by a **bispecific** **antibody** to CD33 and **CD16** (Fc gamma RIII) expressed by human natural killer (NK) cells.

Silla LM; Chen J; Zhong RK; Whiteside TL; Ball ED
Division of Hematology/Bone Marrow Transplantation, University of Pittsburgh School of Medicine, Pennsylvania, USA.

Br J Haematol (ENGLAND) Apr 1995, 89 (4) p712-8, ISSN 0007-1048

Journal Code: AXC

Contract/Grant No.: CA31888, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Bispecific **antibodies** recognizing tumour-associated antigens and trigger molecules expressed on immune effector cells have been shown to redirect cytotoxicity of several types of peripheral blood cells against relevant tumour targets. Among various effector cells, natural killer (NK) cells appear to play a role in defence against leukaemia. Here we report the successful chemical conjugation of monoclonal antibodies to CD33 and **CD16** to create a **bispecific** **antibody** (BsAb 251 x 3G8). This **bispecific** **antibody** is capable of augmenting the killing of otherwise resistant leukaemia cells by peripheral blood lymphocytes (PBL), purified resting NK (R-NK) cells, and activated NK (A-NK) cells. BsAb 251 x 3G8 may play a role in the therapy of acute myeloid leukaemia (AML) through redirecting the cytotoxic activity of endogenous or adoptively transferred NK cells.

23/3,AB/11 (Item 11 from file: 155)
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09326233 95256233

Heterotypic Fc gamma R clusters evoke a synergistic Ca²⁺ response in human neutrophils.

Vossebeld PJ; Kessler J; von dem Borne AE; Roos D; Verhoeven AJ
Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam.

J Biol Chem (UNITED STATES) May 5 1995, 270 (18) p10671-9, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Both **Fc** **gamma** **receptors** on human neutrophils (Fc gamma RIla and Fc gamma RIIfb) are capable of initiating signal transduction after multivalent cross-linking. However, immune complexes most likely activate neutrophils by a combined homotypic and heterotypic cross-linking of Fc gamma Rs. We have investigated the effect of homotypic and heterotypic Fc gamma R cluster formation on changes in the intracellular free Ca²⁺ concentration. Combined heterotypic and homotypic cluster formation resulted in a Ca²⁺ response that was strongly enhanced as compared to the sum of both individual Fc gamma R responses. This synergistic response was caused by the formation of heterotypic clusters of Fc gamma Rs and not by the simultaneous formation of homotypic clusters. This conclusion was supported by experiments with a **bispecific** **antibody** binding to both Fc gamma RIla and Fc gamma RIIfb. The heterotypic Fc gamma R cross-linking results in efficient activation of Ca²⁺ influx, probably caused by a more pronounced depletion of intracellular Ca²⁺ stores. Stimulation with immune complexes also induced Ca²⁺ influx in normal neutrophils, but not in Fc gamma RIIfb-deficient neutrophils. The synergism between both Fc gamma Rs was also apparent in other responses of neutrophils, such as the activation of the respiratory burst. This study shows that the two different Fc gamma Rs on neutrophils complement each other in mediating an important cellular response.

23/3,AB/12 (Item 12 from file: 155)
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09208530 95138530

Interaction of human monocyte **Fc** **gamma** **receptors** with rat IgG2b. A new indicator for the Fc gamma RIla (R-H131) polymorphism.

Haagen IA; Geerars AJ; Clark MR; van de Winkel JG

Department of Immunology, University Hospital Utrecht, The Netherlands.

J Immunol (UNITED STATES) Feb 15 1995, 154 (4) p1852-60, ISSN 0022-1767 Journal Code: IFB

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Rat mAbs receive considerable interest for immunologic intervention in man. The rat IgG2b isotype has previously been found to be optimally active both in vivo and in vitro. We found that both a rat IgG2b CD3 mAb and a monovalent hybrid rat IgG2b-mouse IgG1 bispecific Ab triggered T cell activation in PBMC. Inhibition analyses with mAb blocking different human IgG Fc receptors (Fc gamma R) showed a dimorphic pattern. In donors expressing an Fc gamma RIla-R/R131 allotype (previously defined on the basis of interaction with mouse (m) IgG1 as "high responder") anti-Fc gamma RI mAb 197 inhibited rat IgG2b induced T cell mitogenesis almost completely. In Fc gamma RIla-H/H131 ("low responder" allotype) donors, however, both anti-Fc gamma RI mAb 197 and anti-Fc gamma RII mAb IV.3 were essential for optimal inhibition of mitogenesis. T cell proliferation experiments performed with the use of Fc gamma R-transfected fibroblasts as accessory cells showed the high affinity Fc gamma RIa (CD64) to interact with both rat IgG2b and rat IgG2b-mIgG1 hybrid CD3 mAb. The use of the two types of Fc gamma RIa (CD32)-transfectants instead showed rat IgG2b CD3 mAb to interact solely with the Ila-H/H131 allotype. Interestingly, rat IgG2b-mIgG1 hybrid mAb did not interact effectively with this low affinity Fc gamma R. This suggests a requirement for only one rat IgG2b H chain for Fc gamma RIa-mediated binding, whereas two identical H chains seem to be necessary for proper interaction with Fc gamma RIla. Ab-sensitized RBC-rosette experiments performed with the use of a rat IgG2b anti-NIP mAb

confirmed the interaction pattern observed with rat CD3 mAb, supporting the phenomena to be isotype-, and not mAb-, dependent. These analyses point to a unique reactivity pattern for rat IgG2b Abs, interacting both with the high affinity Fc gamma RIa in all donors and Fc gamma RIa of individuals expressing the IIa-H131 allotype.

23/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09005116 94320116

Antigen forks: bispecific reagents that inhibit cell growth by binding selected pairs of tumor antigens.

Ring DB, Hsieh-Ma ST; Shi T; Reeder J
Department of Immunotherapeutics, Chiron Corporation, Emeryville, CA 94608.

Cancer Immunol Immunother (GERMANY) Jul 1994, 39 (1) p41-8, ISSN 0340-7004 Journal Code: CN3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Bispecific **antibodies** of a new category, termed "antigen forks", were constructed by crosslinking antibodies that recognized pairs of distinct tumor cell surface antigens. At concentrations of 1-100 nM, several such forks inhibited the growth of human tumor cell lines bearing both relevant antigens. The same cells were not inhibited by unconjugated component antibodies, and the active conjugates did not inhibit the growth of human cell lines that expressed lower levels of relevant antigens. The three most active antigen forks all contained monoclonal antibody 454A12, which recognizes human transferrin receptor. This antibody was conjugated respectively to antibodies 113F1 (against a tumor-associated glycoprotein complex), 317G5 (against a 42-kDa tumor-associated glycoprotein), or 520C9 (against the c-erbB-2 protooncogene product). The 317G5-454A12 fork strongly inhibited the HT-29 and SW948 human colorectal cancer cell lines, while the 113F1-454A12 and 520C9-454A12 forks strongly inhibited the SK-BR-3 human breast cancer cell line and the 113F1-454A12 fork was also effective against SW948. By designing forks against antigens of incompatible function that are co-expressed at high levels on tumor cells but not on normal tissues, it may be possible to generate reagents that inhibit tumor growth with enhanced selectivity.

23/3,AB/14 (Item 14 from file: 155)
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08938701 94253701

Role of **Fc** **gamma** **receptors** in cancer and infectious disease.

Wallace PK; Howell AL; Fanger MW
Department of Microbiology, Dartmouth Medical School, Lebanon, New Hampshire 03756.

J Leukoc Biol (UNITED STATES) Jun 1994, 55 (6) p816-26, ISSN 0741-5400 Journal Code: IWY

Contract/Grant No.: AI 19053, AI, NIAID; AI 31800, AI, NIAID; CA 23108, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW, ACADEMIC

Receptors for the Fc domain of immunoglobulin G (Fc gamma R) provide an interface between specific humoral immunity and the cellular branch of the immune system through their interaction with antibody. Cross-linking Fc gamma R on myeloid cells triggers such diverse functions as clearance of immune complexes, phagocytosis of opsonized pathogens, secretion of reactive oxygen intermediates, and antibody-dependent cellular cytotoxicity. The Fc gamma R play a major role in the removal of antibody-coated infectious agents and are the exclusive trigger molecules for tumor cell killing by human myeloid cells. Studies of Fc gamma R function have been aided by the use of Fc gamma R specific monoclonal antibodies, self-directed target cells, and **bispecific** **antibodies** that link target cells or pathogens to specific host cell molecules, including Fc gamma R. These reagents have contributed significantly to our understanding of the role of the different classes of Fc gamma R in mediating protection from various infectious agents and in mediating tumor

cell killing. Taken together, these approaches have provided insight into the utility of manipulating Fc gamma R function in the therapy of cancer and infectious disease.

23/3,AB/15 (Item 15 from file: 155)
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08900205 94215205

Potential of long-term-cultured lymphokine-activated killer cell cytotoxicity against small-cell lung carcinoma by anti-CD3 x anti-(tumor-associated antigen) ****bispecific**** ****antibody****.

Azuma A; Yagita H; Okumura K; Kudoh S; Niitani H
4th Department of Internal Medicine, Nippon Medical School, Japan.
Cancer Immunol Immunother (GERMANY) May 1994, 38 (5) p294-8, ISSN 0340-7004 Journal Code: CN3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Lymphokine-activated killer (LAK) cells exhibit a potent cytotoxicity to malignant cells in vitro. However, a satisfactory effect has not been obtained in many clinical studies except for a few cases. One of the most important reasons why cytolytic activity could not be exhibited in vivo is that LAK cells do not accumulate in the tumor tissue because of a lack of specificity. In the present study, we show the effect of a ****bispecific**** ****antibody**** (bsAb) on the accumulation of LAK cells around the small-cell lung carcinoma (SCLC) cell and the subsequent enhancement of LAK cell cytotoxicity against SCLC. When short-term(4 days)-cultured LAK cells were used, OKT3 x LU246 bsAb, which direct CD3+ T-LAK cells to the target cell, induced a similar level of cytotoxicity to that induced by 3G8 x LU246 bsAb, which directs ****CD16****+ LAK cells. Long-term(21 days)-cultured LAK cells exhibited a reduced spontaneous cytotoxicity but retained high cytotoxic activity, which could be directed by OKT3 x LU246 or 3G8 x LU246 bsAb. The inhibitory effect of LAK cells on tumor cell clonogenicity in soft agar was also enhanced by both bsAb. These results indicate that application of the therapy with LAK cells and OKT3 x LU246 bsAb to SCLC patients might be a promising new method of adoptive immunotherapy.

23/3,AB/16 (Item 16 from file: 155)
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08871659 94186659

Immunohistochemical detection of c-erbB-2 expression by neoplastic human tissue using monospecific and bispecific monoclonal antibodies.

Garcia de Palazzo I; Klein-Szanto A; Weiner LM
Fox Chase Cancer Center, Philadelphia, PA 19111.
Int J Biol Markers (ITALY) Oct-Dec 1993, 8 (4) p233-9, ISSN 0393-6155
Journal Code: IJM

Contract/Grant No.: CA06927, CA, NCI; CA50633, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Selected murine monoclonal antibodies (MAb) have been shown to inhibit relevant tumor growth in vitro and in animal models. Recently, ****bispecific**** ****antibodies**** (BsMAb) have been developed which target cytolytic effector cells via one antibody binding site and tumor antigen by the other specificity. For example, the BsMAb 2B1 possesses specificity for c-erbB-2 and Fc gamma RIII, the low affinity ****Fc**** ****gamma**** ****receptor**** expressed by polymorphonuclear leukocytes (PMN), macrophages and large granular lymphocytes (LGL). The human homologue of the rat neu oncogene, c-erbB-2, has been demonstrated to be amplified in breast, gastrointestinal, lung and ovarian carcinomas. Tumor expression of c-erbB-2 has been shown to be an important prognostic indicator in breast and ovarian carcinomas. The restricted expression of the c-erbB-2 protooncogene product in normal human tissues and the wide distribution of c-erbB-2 expression in such tumors may justify attempts to use an appropriately constructed BsMAb in clinical trials. In this report we have addressed this issue by immunohistochemically evaluating the expression of c-erbB-2 oncogene product in a variety of malignant tumors utilizing 2B1 and the anti-c-erbB-2 monovalent parent of 2B1, 520C9. Among the studied neoplasms, c-erbB-2 expression was detected in 49% of primary carcinomas stained with

520C9 and in 39% of those stained with 2B1. In the group of metastatic tumors, c-erbB-2 oncoprotein was detected in 52% of cases by 520C9 and in 41% by 2B1.(ABSTRACT TRUNCATED AT 250 WORDS)

23/3,AB/17 (Item 17 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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08863820 94178820

Promotion of natural killer cell growth in vitro by bispecific (anti-CD3 x anti-**CD16**) antibodies.

Malygin AM; Somersalo K; Timonen T

Department of Pathology, University of Helsinki, Finland.

Immunology (ENGLAND) Jan 1994, 81 (1) p92-5, ISSN 0019-2805

Journal Code: GH7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Bispecific heteroconjugated F(ab')₂ fragments were prepared from pepsin-digested monoclonal OKT3 (anti-CD3) and 3G8 (anti-**CD16**) antibodies with 5,5'-dithiobis- (2-nitrobenzoic acid). When these **bispecific** **antibodies** (BsA) were added to peripheral blood lymphocyte (PBL) cultures with 100 U/ml human recombinant interleukin-2 (rIL-2), preferable growth of natural killer cells occurred. After 3 weeks the frequencies of CD56+ and CD56+3- cells in cultures with BsA were 74 +/- 7% and 65 +/- 7%, respectively, compared with 48 +/- 6% and 29 +/- 7% in control cultures. The frequencies of CD3+ lymphocytes in the presence of BsA, cells from 1-day cultures were labelled with fluorescein isothiocyanate (FITC)-conjugated anti-CD3, CD4 and CD8 monoclonal antibodies (mAb) and propidium iodide which stains dead cells. Flow cytometry revealed that more than 95% of the dead cells in cultures with BsA were CD3+. Thirty-seven per cent of CD3+, 43% of CD4+ and 17% of CD8+ cells were dead on day 1, and after 3 days the CD4+/CD8+ ratio among viable lymphocytes was 1.6 in the control and 0.5 in BsA cultures. Taken together, these results show that bispecific (anti-CD3 x anti-**CD16**) F(ab')₂ fragments are strongly immunomodulatory by inducing the killing of T cells by **CD16** cells.

23/3,AB/18 (Item 18 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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08749175 94064175

A **CD16**/CD30 bispecific monoclonal antibody induces lysis of Hodgkin's cells by unstimulated natural killer cells in vitro and in vivo.

Hombach A; Jung W; Pohl C; Renner C; Sahin U; Schmits R; Wolf J; Kapp U; Diehl V; Pfreundschuh M

Medizinische Klinik, Universitat des Saarlandes, Homburg/Saar, Germany.

Int J Cancer (UNITED STATES) Nov 11 1993, 55 (5) p830-6, ISSN

0020-7136 Journal Code: GQU

Languages: ENGLISH

Document type: JOURNAL ARTICLE

In order to target NK cells against the Hodgkin's-derived cell line L540, we developed bispecific monoclonal antibodies (Bi-MABs) by somatic hybridization of the 2 mouse hybridoma cell line HRS-3 and A9 which produce monoclonal antibodies (MABs) with reactivity against the Hodgkin and Reed-Sternberg cell-associated CD30 antigen and the **CD16** antigen (Fc gamma III receptor), respectively. The **CD16** MAB-producing cell line A9 was selected as a partner for HRS-3 because of its efficiency in inducing lysis of the A9 hybridoma cells by resting NK cells. The hybrid hybridoma cell line HRS-3/A9 produced the supernatant with the strongest bispecific reactivity and was repeatedly subcloned and used for ascites production. Crude supernatant and purified HRS-3/A9 Bi-MAB triggered specific lysis of the CD30+ Hodgkin's-derived cell line L540, but not of the CD30- cell line HPB-ALL by unstimulated peripheral-blood lymphocytes and NK-cell-enriched populations. Moreover, treatment of SCID mice bearing heterotransplanted human Hodgkin's tumors with HRS-3/A9 and human peripheral blood lymphocytes induced specific complete tumor regression in 10/10 animals. We thus report successful tumor treatment in an in vivo model using NK-cell-associated Bi-MABs and show that the Bi-MAB HRS-3/A9 is an efficient promoter of the anti-tumor effects of NK cells in vitro and in vivo.

23/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08691336 94006336

Use of anti-CD3 and anti-**CD16** bispecific monoclonal antibodies for the targeting of T and NK cells against tumor cells.

Ferrini S; Cambiaggi A; Sforzini S; Canevari S; Mezzanzanica D; Colnaghi MI; Moretta L

Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy.

Cancer Detect Prev (UNITED STATES) 1993, 17 (2) p295-300, ISSN 0361-090X Journal Code: CNZ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

To target T lymphocytes against EGF-R+ tumors, we constructed anti-CD3/anti-EGF-R bimAbs either by the generation of a hybrid hybridoma (quadroma) or by a chemical cross-linking method. Analysis of the in vitro functional activity of these two different constructs indicated that the quadroma-secreted bimAb was more efficient in targeting the CD3+8+ clones against EGF-R+ target cells with respect to the bimAb produced by chemical method. In addition, the quadroma-produced bimAb is able to induce cytotoxicity of EGF-R+ tumor cell lines of PHA-induced lymphoblasts that had been expanded in IL-2-containing medium, whereas tumor cells lacking expression of EGF-R were not lysed. Resting PBL targeted by the bimAb did not display significant cytotoxicity against the relevant tumor. An anti-**CD16** hybridoma (IgG1) was fused with an anti-folate-binding protein hybrid (IgG2a) to construct bimAbs to target NK cells against NK-resistant ovarian carcinomas. The hybrid IgG1/IgG2a bimAb triggered the specific lysis of relevant target cells by resting NK cells, but it was ineffective when CD8+TCR alpha/beta+ cultured cell populations were used as effectors. Only marginal increases of cytolytic activity could be induced by the bimAb when IL-2-activated PBL (i.e., LAK cells) were used as effectors due to the high cytolytic activity of these cells against the relevant tumors in the absence of bimAb. The possible use of anti-**CD16** or anti-CD3 bimAbs for the development of different cellular immunotherapy strategies against cancer is discussed.

23/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08623105 93333105

Involvement of the high-affinity receptor for IgG (Fc gamma RI; CD64) in enhanced tumor cell cytotoxicity of neutrophils during granulocyte colony-stimulating factor therapy.

Valerius T; Repp R; de Wit TP; Berthold S; Platzer E; Kalden JR; Gramatzki M; van de Winkel JG

Department of Medicine III, University of Erlangen-Nurnberg, Germany.

Blood (UNITED STATES) Aug 1 1993, 82 (3) p931-9, ISSN 0006-4971

Journal Code: A8G

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Three different classes of Fc receptors for IgG (Fc gamma R) are currently distinguished in humans, of which polymorphonuclear phagocytes (PMN) normally express both low-affinity receptor classes--Fc gamma RII (CD32) and Fc gamma RIII (**CD16**). During therapy with granulocyte colony-stimulating factor (G-CSF), neutrophils from patients with various malignancies and different hematologic disorders were found to additionally express high levels of the receptor with high affinity for IgG (Fc gamma RI; CD64). For these patients, the relative fluorescence intensity (rFI) for Fc gamma RI was 5.3 (range, 1.7 to 10.3; n = 19), compared with 1.0 (range, 1.0 to 1.1; n = 8) for healthy donors. The expression of Fc gamma RI during G-CSF therapy could be confirmed by using a panel of six CD64-specific antibodies, and by showing mRNA for Fc gamma RI. So far, three genes for Fc gamma RI have been identified, encoding four distinct transcription products. By reverse transcriptase-polymerase chain reaction technology, transcripts for both membrane-associated isoforms (hFc gamma RIa and hFc gamma RIb2) could be detected. The functional activity of Fc gamma RI on PMN during G-CSF therapy was shown by measuring binding of

monomeric human IgG and antibody-dependent cellular cytotoxicity (ADCC). Thus, Fc gamma RI-positive neutrophils displayed enhanced ADCC activity to glioma (A1207), squamous cell (A431), and ovarian (SK-ov3) carcinoma cell lines. The involvement of Fc gamma RI in this increased cytotoxic activity was shown by blocking ****Fc** **gamma** **receptors**** with monoclonal antibodies, and by using F(ab')₂ x F(ab')₂-****bispecific** **antibodies**** with specificities against tumor-related antigens and Fc gamma RI, resulting in solely Fc gamma RI-mediated cytotoxicity. Therapeutically, this additional Fc receptor on PMN may increase the efficacy of experimental antibody therapy.

23/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08550397 93260397

Neuraminidase augments ****Fc** **gamma** **receptor**** II-mediated antibody-dependent enhancement of dengue virus infection.

Mady BJ; Kurane I; Erbe DV; Fanger MW; Ennis FA
Department of Medicine, University of Massachusetts Medical Center, Worcester 01655.

J Gen Virol (ENGLAND) May 1993, 74 (Pt 5) p839-44, ISSN 0022-1317
Journal Code: I9B

Contract/Grant No.: RO1-AI30624, AI, NIAID; T32-AI07272, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Antibody-dependent enhancement (ADE) of dengue virus infection occurs when neutralizing antibodies at sub-neutralizing concentrations or non-neutralizing antibodies form complexes with the virus. These virus-antibody complexes can then attach to a ****Fc** **gamma** **receptor****-bearing cell, via the Fc portion of the immunoglobulin, resulting in an increased number of infected cells. ADE may be responsible in part for the most severe clinical manifestations of dengue virus infection which include haemorrhage and shock. Three classes of human ****Fc** **gamma** **receptors**** exist, Fc gamma RI, Fc gamma RII and Fc gamma RIIL. In this study, we examined the effects of neuraminidase on ADE of dengue virus infection mediated by the low-affinity Fc gamma RIIL. K562 cells, which express only Fc gamma RII, treated with neuraminidase resulted in augmentation of ADE of dengue virus infection by human anti-dengue antibodies. This augmented ADE of infection could be blocked by anti-Fc gamma RII monoclonal antibody IV.3. Incubation of neuraminidase-treated K562 cells with IgG-coated human red blood cells resulted in an increase in the percentage of rosette formations compared with the untreated K562 cells. A ****bispecific** **antibody**** directed against Fc gamma RII and dengue virus (IV.3 x 2H2) enhanced virus infection. Neuraminidase also augmented ADE mediated by this antibody, but to a much lesser degree (by 50%) compared with that seen using conventional human anti-dengue antibody (by 200 to 300%). Fluorescence-activated cell sorting analysis of neuraminidase-treated K562 cells showed that the number of Fc gamma RII-specific antibodies that bind to Fc gamma RII increases by 15 to 20% after treatment with neuraminidase. These results indicate that neuraminidase augments ADE of dengue virus infection and that the augmented ADE is mediated through Fc gamma RII.

23/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08467744 93177744

The role of apoptosis in antibody-dependent cellular cytotoxicity.

Curnow SJ; Glennie MJ; Stevenson GT
Lymphoma Research Unit, Tenovus Laboratory, General Hospital, Southampton, UK.

Cancer Immunol Immunother (GERMANY) 1993, 36 (3) p149-55, ISSN 0340-7004
Journal Code: CN3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Apoptosis in three lymphoma cell lines has been studied following cytotoxicity induced in vitro by normal human blood lymphocytes utilizing either natural killer (NK) or antibody-dependent cellular cytotoxic (ADCC)

mechanisms. Guinea-pig L2C leukaemic lymphocytes, but not the human cell lines Daudi and Jurkat, revealed a degree of time- and temperature-dependent apoptotic death upon simple culture in vitro. NK cytotoxicity at low effector:target ratios (E:T) induced both release of ⁵¹Cr and apoptosis. However NK cytotoxicity at higher E:T, and ADCC at all E:T, increased the level of ⁵¹Cr release while reducing the level of apoptosis. The findings were consistent with the apoptotic process being cut short by intervention of necrotic death. The same characteristics accompanied ADCC whether the effectors were recruited by Fc gamma regions of antibody coating the targets, or by ****bispecific** **antibodies**** attaching one arm to the targets and the other to ****Fc** **gamma** **receptors**** type III on effectors. This finding, and the high level of cytotoxicity elicited by the bispecific method, confirm the belief that NK cells, in addition to exerting NK cytotoxicity, represent the principal effectors for ADCC among blood mononuclear cells. Our results suggest that NK cells have both apoptotic and necrotic mechanisms available for killing their targets, but use only the latter for ADCC.

23/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08453666 93163666
****Fc** **gamma** **receptors**** in cancer and infectious disease.
Fanger MW; Erbe DV
Department of Microbiology, Dartmouth Medical School, Lebanon, N.H. 03756.
Immunol Res (SWITZERLAND) 1992, 11 (3-4) p203-16, ISSN 0257-277X
Journal Code: IMR
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
Through interaction with antibody, IgG Fc receptors provide an interface between specific humoral immunity and Fc gamma R-bearing host cells. Fc gamma R trigger such diverse functions as immune complex clearance, phagocytosis of opsonized pathogens, reactive oxygen intermediate and enzyme secretion, and antibody-dependent cellular cytotoxicity (ADCC). Moreover, Fc gamma R are the exclusive trigger molecules for tumor cell killing by human myeloid cells. Studies of Fc gamma R function have been aided by the use of ****bispecific** **antibodies**** to link cells or pathogens to specific host cell molecules, including Fc gamma R. These reagents have permitted determination of the role of Fc gamma R in ADCC of the protozoan, *Toxoplasma gondii*, by human effector cells. This approach has also indicated that Fc gamma R do not serve as entry points for viruses such as dengue virus and HIV. Taken together, these results provide insight into the utility of manipulating Fc gamma R function in the therapy of cancer and infectious disease.

23/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08382128 93092128
In vitro cytotoxic targeting by human mononuclear cells and ****bispecific** **antibody**** 2B1, recognizing ****c**-**erbB****-2 protooncogene product and ****Fc** **gamma** **receptor**** III.
Hsieh-Ma ST; Eaton AM; Shi T; ****Ring DB****
Chiron Corporation, Emeryville, California 94608.
Cancer Res (UNITED STATES) Dec 15 1992, 52 (24) p6832-9, ISSN 0008-5472 Journal Code: CNF
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Bispecific murine monoclonal antibody 2B1, possessing dual specificity for the human ****c**-**erbB****-2 protooncogene product and human ****Fc** **gamma** **receptor**** III (****CD16****) was evaluated for the ability to promote specific lysis of ****c**-**erbB****-2-positive tumor cells in vitro. In short-term ⁵¹Cr release assays with human mononuclear cells as effectors and SK-Br-3 human breast cancer cells as targets, neither parental antibody of 2B1 mediated significant specific lysis, but ****bispecific** **antibody**** was as active as a chemical heteroconjugate, with 5 ng/ml of 2B1 causing half-maximal lysis at an effector/target ratio of 20:1 and 2 ng/ml 2B1

causing half-maximal lysis at an E/T ratio of 40:1. The cytotoxic targeting activity of 2B1 F(ab)₂ fragment was the same as that of whole ****bispecific** **antibody****, and the activity of whole 2B1 was not reduced when assays were performed in 100% autologous human serum, indicating that 2B1 binds effector cells through the ****CD16****-binding site derived from parental antibody 3G8 rather than through its Fc portion. Variable inhibition of 2B1-mediated lysis was observed when autologous polymorphonuclear leukocytes from different donors were added to mononuclear effector cells at a 2:1 ratio; this inhibition was overcome at higher antibody concentration. 2B1 bispecific monoclonal antibody was also able to mediate targeted cytotoxicity using whole human blood as a source of effector cells or using effector or target cells derived from ovarian cancer patients.

23/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08297954 93007954

Antitumor effects of a ****bispecific** **antibody**** targeting CA19-9 antigen and ****CD16****.

Garcia de Palazzo I; Holmes M; Gercel-Taylor C; Weiner LM
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.

Cancer Res (UNITED STATES) Oct 15 1992, 52 (20) p5713-9, ISSN 0008-5472 Journal Code: CNF

Contract/Grant No.: CA06927, CA, NCI; CA50633, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Bispecific murine monoclonal antibodies that target tumor and Fc gamma RIII (****CD16****) can promote relevant tumor lysis by large granular lymphocytes. For these antibodies to be clinically useful, their properties should be maintained in vivo, where competing human immunoglobulin, shed target antigen, and shed ****CD16**** may be encountered. At a minimum, ****bispecific** **antibody**** antitumor effects should be preserved in whole blood. Furthermore, potentiation of tumor lysis should be reflected by demonstrating the ability of ****bispecific** **antibody**** -retargeted effector cells to infiltrate and mediate lysis of organized tumor. If these characteristics are demonstrated, and there is evidence of in vivo efficacy of ****bispecific** **antibody**** -based therapy in a relevant animal model, further clinical development of such antibodies would be warranted. In this report the ability of CL158 ****bispecific** **antibody**** supernatants to mediate lysis of SW948 tumor growing in monolayer is shown to be preserved in the presence of interleukin 2-activated whole blood. When SW948 cells were grown in vitro as multicellular human tumor spheroids, incubation with interleukin 2-activated lymphocytes (LAK cells) and CL158 led to structural and widespread necrosis. This was dependent on CL158 and resistant to competition by pooled human immunoglobulin or interleukin 2-exposed whole blood. These effects were not promoted by the monospecific antibodies produced by the parent clones of CL158 and were not observed when the IgG2a variant of CA19-9 antibody, which mediates conventional antibody-dependent cellular cytotoxicity, was used instead of its bispecific derivative. To examine the efficacy of ****bispecific** **antibody**** -based treatments on in vivo tumor, scid mice bearing early s.c. SW948 xenografts were treated with interleukin 2 for 5 consecutive days, supplemented by three i.v. injections of 10(7) human LAK cells and various antibodies. Treatment of mice bearing SW948 tumors with LAK cells did not retard tumor growth, but when CL158 was added, significant delays in tumor growth were observed. Tumor growth delay required treatment with both LAK cells and the ****bispecific** **antibody****. Treatment with the IgG2a variant of CA19-9 antibody, alone or with LAK cells, had no effects on tumor growth. Although the mechanisms of these antitumor effects require further study, it is clear that human LAK cell treatment of animals bearing early, established s.c. tumors is enhanced by the addition of ****bispecific** **antibodies**** with relevant binding characteristics. When compared with the IgG2a isotype variant of CA19-9 monoclonal antibody, this ****bispecific** **antibody**** offers the advantages of preservation of activity in physiological conditions, infiltration and disruption of organized tumor in vitro, and antitumor effects in a relevant xenograft model.(ABSTRACT TRUNCATED AT 400 WORDS)

23/3,AB/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08292914 93002914

Redirected targeting of LDL to human monocyte **Fc** **gamma**
receptors with **bispecific** **antibodies**.

Morganelli PM; Kitzmiller TJ; Hemmer R; Fanger MW
Veterans Administration Hospital, Research Service, White River Junction,
VT 05009.

Arterioscler Thromb (UNITED STATES) Oct 1992, 12 (10) p1131-8, ISSN
1049-8834 Journal Code: AZ1

Contract/Grant No.: AI-19053, AI, NIAID; CA-44794, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Recent studies indicate that low density lipoprotein (LDL)-immune complexes consisting of anti-LDL antibodies bound to LDL may contribute to macrophage foam cell development by uptake through immunoglobulin G (IgG) Fc receptors. As human mononuclear phagocytes possess three structurally and functionally distinct classes of IgG Fc receptors, we developed a system whereby the effects of LDL-immune complexes could be studied with respect to each type of IgG Fc receptor. Novel **bispecific** **antibodies** consisting of anti-**Fc** **gamma** **receptor** antibodies linked to anti-LDL antibodies were used to prepare bispecific LDL-immune complexes for targeting to specific **Fc** **gamma** **receptors**. In this report, the effects of bispecific LDL-immune complexes directed to **Fc** **gamma** **receptor** types I, II, and III were studied primarily with monocytes and were compared with the effects of similarly prepared bispecific complexes that targeted LDL to human leukocyte antigen (HLA) class I antigens. Each type of **bispecific** **antibody** was effective in targeting 125I-LDL to its respective site on the cell surface. Using fluorophore-labeled LDL and flow cytometry, bispecific complexes directed to **Fc** **gamma** **receptor** types I or II but not to HLA class I antigens caused a two- to sevenfold increase in cell-associated fluorescence relative to control cells treated with LDL in the absence of **bispecific** **antibody**. Uptake occurred in the presence of excess unlabeled LDL, acetylated LDL, and antioxidants. That the bispecific complexes triggered metabolic uptake was supported by studies of kinetics and temperature dependence. Using 125I-labeled complexes, metabolic degradation of LDL was demonstrated in association with each of the three types of **Fc** **gamma** **receptors**. (ABSTRACT TRUNCATED AT 250 WORDS)

23/3,AB/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08228926 92366926

Cytoplasmic calcium fluxes induced in cytotoxic effector cells by engagement of **Fc** **gamma** **receptors** I, II, and III.

Curnow SJ; Glennie MJ; Stevenson GT
Lymphoma Research Unit, Tenovus Laboratory, General Hospital,
Southampton, UK.

Scand J Immunol (ENGLAND) Aug 1992, 36 (2) p221-31, ISSN 0300-9475
Journal Code: UCW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Changes in the intracellular calcium ion concentration ([Ca²⁺]_i) of monocytes, granulocytes, and NK cells have been studied following either (1) independent cross-linking of **Fc** **gamma** **receptors** (Fc gamma R) I, II, or III, with F(ab gamma)₂ fragments of monoclonal antibodies; or (2) linking of a selected Fc gamma R to a tumour cell target with bispecific F(ab' gamma)₂ antibodies. Upon cross-linking each Fc gamma R with antibody an increase in the [Ca²⁺]_i was observed, although all receptors apart from Fc gamma RIII on NK cells required additional cross-linking with an anti-mouse Fab' gamma. These results indicate that each type of receptor can transduce signals to the cell independently. **Bispecific** **antibodies** (anti-Fc gamma R x anti-target) linking cytotoxic Fc gamma R-bearing effector cells to tumour target cells also mediated increases in [Ca²⁺]_i for all Fc gamma R tested except for Fc gamma RIII on granulocytes. The failure to transduce a signal via this receptor may be related to the GPI link, which is in contrast to the transmembrane

link of Fc gamma RIII on NK cells. Significant lysis of tumour cell targets occurred when ****bispecific**** ****antibodies**** recruited NK cells or monocytes, but not granulocytes, via Fc gamma R. Chelation of intracellular Ca2+ in the effector cells reduced the observed lysis, suggesting a role for Ca2+ in the pathways leading to cytotoxicity.

23/3,AB/28 (Item 28 from file: 155)
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08086191 92224191

Comparative efficiencies of bispecific F(ab'gamma)2 and chimeric mouse/human IgG antibodies in recruiting cellular effectors for cytotoxicity via ****Fc**** ****gamma**** ****receptors****.
Greenman J; Hogg N; Nikolett S; Slade C; Stevenson G; Glennie M
Tenovus Research Laboratory, General Hospital, Southampton, UK.
Cancer Immunol Immunother (GERMANY) 1992, 34 (6) p361-9, ISSN 0340-7004 Journal Code: CN3
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The three forms of ****Fc**** ****gamma**** ****receptor**** carried by monocytes (Fc gamma RI, II) and natural killer (NK) cells (Fc gamma RIII) are all capable of mediating cell lysis. Here we compare the use of F(ab'gamma)2 ****bispecific**** ****antibodies****, specifically targetting individual Fc gamma R, and chimeric IgG mouse/human antibodies which are capable of targetting all Fc gamma R, for their ability to mediate target cell destruction. The derivatives are prepared by linking hinge sulphhydryl residues via tandem thioether bonds, using a bismaleimide crosslinker: Fab' from an anti-Fc gamma R mAb linked to Fab' from a common anti-target mAb (BsAb), or Fab' from the common anti-target mouse antibody linked to human Fc gamma (FabFc or bisFabFc). All the derivatives targetting chick red blood cells gave efficient lysis, although different effector cell donors yielded differences in both the lytic levels achieved and the comparative efficiencies of derivatives. In contrast, significant lysis of the guinea pig lymphoblastic leukaemia, L2C, regularly resulted only via the anti-Fc gamma RIII BsAb and the chimeric derivatives. These results suggest that the chimeric, Fc-containing derivatives mediate tumour cell lysis principally through Fc gamma RIII on NK cells. This is in contrast to the situation with the chick red blood cells where the chimeric derivatives appear capable of lysing erythrocytes by utilizing either monocytes or NK cells, because significant (approximately 50%) lysis occurred with effector cell populations magnetically depleted through either Fc gamma RI or Fc gamma RIII. A major difference between these two types of antibody derivative was their ability to function in the presence of high concentrations of normal human Fc gamma. The lysis mediated by BsAb reactive with Fc gamma RI or II was unaffected by the presence of human Fc gamma at 2.5 mg/ml (a concentration comparable with that yielded by IgG in plasma) whereas the BsAb recognizing Fc gamma RIII and all the Fc-containing derivatives were completely inhibited.

23/3,AB/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08060788 92198788

A ****bispecific**** ****antibody**** detects cytotoxic T lymphocytes of unknown antigen specificity in patients with granular lymphocyte-proliferative disorders.

Kaneko T; Oshimi K; Seto T; Okumura K; Mizoguchi H
Department of Medicine, Tokyo Women's Medical College.
Br J Haematol (ENGLAND) Feb 1992, 80 (2) p151-6, ISSN 0007-1048
Journal Code: AXC

Languages: ENGLISH
Document type: JOURNAL ARTICLE

A simple and sensitive method for the detection of cytotoxic T lymphocytes (CTL) of unknown antigen specificity was investigated. Using anti-CD3 Fab' x anti-CD10 Fab' ****bispecific**** ****antibody**** or intact anti-CD3 monoclonal antibody, we induced cytotoxicity for CD10+, ****Fc**** ****gamma**** ****receptor****-positive Daudi target cells in peripheral blood mononuclear cells (PBMC) obtained from patients with granular

lymphocyte-proliferative disorders (GLPD). The results indicated that the ****bispecific**** ****antibody**** was much more efficient than the intact anti-CD3 monoclonal antibody in inducing cytotoxicity. Since CD3+CD4-CD8+ granular lymphocytes in patients with GLPD are considered to be in vivo-primed CTL of unknown antigen specificity, this ****bispecific**** ****antibody**** method may be useful for detecting in vivo-primed CTL within PBMC. By using this ****bispecific**** ****antibody****, it will be possible to detect circulating in vivo-primed CTL in other clinical conditions.

23/3,AB/30 (Item 30 from file: 155)
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07983371 92121371

[The enhancement of cytolytic activity in lymphokine activated killer cells using bispecific F(ab')₂]

Azuma A

Department of Clinical Pathology, Nippon Medical School, Tokyo, Japan.
Nippon Ika Daigaku Zasshi (JAPAN) Dec 1991, 58 (6) p663-72, ISSN 0048-0444 Journal Code: HRD

Languages: JAPANESE Summary Languages: ENGLISH

Document type: JOURNAL ARTICLE English Abstract

In this study, we show that bispecific hetero-F(ab')₂ enhances cytolytic activity in human lymphokine activated killer (LAK) cells derived from peripheral blood mononuclear cells against human small cell lung cancer (SCLC) cell lines. We used two types of bispecific F(ab')₂ (anti-CD3-LU246mAb, anti-****CD16****-LU246mAb) which play different roles in the enhancement of cytolytic activity in LAK cells against the human SCLC cell lines N231, H69 and Lu135. Anti-CD3 Fab' or anti-****CD16**** Fab' were coupled with LU246 Fab' that recognized the antigen on human SCLC cells for reproducing bispecific F(ab')₂. Anti-****CD16**** (3G8) Fab' conjugated with LU246 Fab' targeted NK-LAK cells to SCLC cells to mediate cytotoxicity, but NK-LAK cells induced by LGL-enriched fraction did not display enhanced cytotoxicity even when ****bispecific**** ****antibody**** was used in 51Cr release assay. Anti-CD3 (OKT3) Fab' conjugated with LU246 Fab' cross-linked T-LAK cells to SCLC cells activated T-LAK cells through the CD3 complex, and enhanced the cytolytic activity of T-LAK cells against SCLC lines. Although OKT3-LU246 F(ab')₂ was not so potent in enhancing cytolytic activity in 51Cr release assay, it played a greater role in enhancing the inhibitory effect on tumor growth than 3G8-LU246 F(ab')₂ in human tumor clonogenic assay and in vivo tumor neutralization assay. In addition, the enhancement of target cell lysis by ****bispecific**** ****antibodies**** was generally more potent than antibody dependent cellular cytotoxicity (ADCC) using LU246 monoclonal antibody.

23/3,AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07954455 92092455

[Bi-specific F(ab')₂ enhances cytolytic activities of LAK cells against human small cell lung cancer (SCLC)]

Azuma A; Niitani H

Department of Pulmonary Disease, Nippon Medical School.
Nippon Kyobu Shikkan Gakkai Zasshi (JAPAN) Sep 1991, 29 (9) p1132-7, ISSN 0301-1542 Journal Code: KQD

Languages: JAPANESE Summary Languages: ENGLISH

Document type: JOURNAL ARTICLE English Abstract

In order to enhance cytolytic activity of lymphokine activated killer (LAK) cells against human small cell lung cancer (SCLC), bispecific F(ab')₂ was used with human LAK cells and human SCLC cell lines (i.e. N231 and H69) as targets. For the construction of bispecific F(ab')₂, LU246 monoclonal antibody (mAb) recognizing the antigen expressed on the surface of both SCLC cell lines, was employed. OKT3 or 3G8 mAbs recognizing CD3 on T cell and ****CD16**** on NK cell functional molecules respectively, were chemically cross-linked to LU246 mAb using 5,5'-dithio-bis 2-nitrobenzoic acid (DTNB). Bispecific F(ab')₂ adding to LAK cells increased the conjugation with target cells. The phenotypes of LAK cells change at their various culture times. Although cytolytic activity of LAK cells gradually decreases after one week of culture time, the enhanced cytotoxicity of LAK cell using

bispecific F(ab)₂ has been kept even after long culture. Furthermore, target cell lysis enhanced by bispecific F(ab)₂ has been greater than that by classical ADCC using LU246 mAb. These ****bispecific** **antibodies**** should be effective reagents for adoptive immunotherapy in human SCLC patients.

23/3,AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07875144 92013144

Antibody-dependent enhancement of dengue virus infection mediated by ****bispecific** **antibodies**** against cell surface molecules other than ****Fc** **gamma** **receptors****.

Mady BJ; Erbe DV; Kurane I; Fanger MW; Ennis FA
Department of Medicine, University of Massachusetts, Medical Center,
Worcester 01655.

J Immunol (UNITED STATES) Nov 1 1991, 147 (9) p3139-44, ISSN
0022-1767 Journal Code: IFB

Contract/Grant No.: T32-AI07272, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

It is known that antibodies to dengue viruses at subneutralizing concentrations enhance dengue virus infection of Fc gamma R+ cells. This phenomenon called antibody-dependent enhancement (ADE) occurs when virus-antibody complexes bind to the Fc gamma R via the Fc portion of the Ig. It has been hypothesized that ADE may be responsible for the pathogenesis of the severe manifestations of dengue virus infection including dengue hemorrhagic fever/dengue shock syndrome. To further analyze the mechanisms of ADE, we prepared ****bispecific** **antibodies**** by chemically cross-linking antidengue virus antibodies to antibodies specific for Fc gamma RI or Fc gamma RII and the non-Fc R molecules beta2 microglobulin, CD15 or CD33 and examined whether these ****bispecific** **antibodies**** could enhance infection. ****Bispecific** **antibodies**** targeting dengue virus to Fc gamma RI or Fc gamma RII enhanced dengue virus infection, consistent with previous reports using conventional antibodies. Furthermore, ****bispecific** **antibodies**** targeting dengue virus to beta2 microglobulin, CD15 or CD33 also enhanced dengue virus infection. ****Bispecific** **antibody**** mediated ADE was inhibited by pretreating the cells with the appropriate blocking mAb. These results indicate that cell surface molecules other than Fc gamma R can mediate ADE and suggest that the Fc gamma R does not provide a unique signal necessary for enhanced infection. We hypothesize that directing dengue virus to the cell surface by a ****bispecific** **antibody**** facilitates the interaction between dengue virus and its receptor, thereby increasing its infectivity.

23/3,AB/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07830601 91349601

Selection of hybrid hybridomas by flow cytometry using a new combination of fluorescent vital stains.

Shi T; Eaton AM; ****Ring DB****

Department of Immunology, Cetus Corporation, Emeryville, CA 94608.

J Immunol Methods (NETHERLANDS) Aug 9 1991, 141 (2) p165-75, ISSN
0022-1759 Journal Code: IFE

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A new combination of fluorescent dyes (rhodamine 123 and hydroethidine) was used to internally label hybridoma fusion partners. Murine hybridoma 520C9 (recognizing human c-erbB-2) was labeled with hydroethidine. Murine hybridoma 3G8 (recognizing human ****Fc** **gamma** **receptor**** III) was labeled with rhodamine 123, and verapamil was used to block rhodamine efflux via P-glycoprotein. Viability assays showed little cytotoxicity from these dyes at the concentrations used. The labeled cells were fused with polyethylene glycol, sorted for dual fluorescence on an Epics V cell sorter, and cloned. Hybrid hybridomas producing ****bispecific** **antibodies**** were selected for ability to promote lysis of SK-Br-3 breast cancer cells by human mononuclear cells. Several positive clones were

obtained and shown to have a double content of DNA. **Bispecific** **antibody** produced by subclone 2B1 was purified by anion exchange chromatography and shown to bind both tumor cells and Fc gamma R III bearing cells. Using two parameter flow cytometric analysis, we were able to measure a 'bridging' effect of this **bispecific** **antibody**, which caused formation of complexes between PMNs and SK-Br-3 cells. Either parental antibody could compete with **bispecific** **antibody** to block such complexing. This fusion method provides several advantages over other techniques presently used (speed, convenience, low toxicity and automatic exclusion of dead cells) and can be applied to produce other hybrid hybridomas.

23/3,AB/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07782741 91301741

Interleukin-2 activated T cells (T-LAK) express **CD16** antigen and are triggered to target cell lysis by **bispecific** **antibody**.
Nitta T; Nakata M; Yagita H; Okumura K
Department of Immunology, Juntendo University School of Medicine, Tokyo, Japan.

Immunol Lett (NETHERLANDS) Apr 1991, 28 (1) p31-7, ISSN 0165-2478
Journal Code: GIH
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Human PBMCs from healthy donors were cultured with 100 U/ml rIL-2 for up to 5 weeks and tested at short and long activation times for the ability to mediate CD3 and **CD16** targeted cytotoxicity using chemically cross-linked **bispecific** **antibodies**. At each period, LAK activity was augmented with the use of **bispecific** **antibodies** (BA), whereas interestingly enough, at later periods (4-5 weeks) when **CD16** positive lymphocytes are not present by flow cytometry, **CD16** targeted cytotoxicity was induced. We suspected the possibility of **CD16** expression on activated T cells and have purified the T cell subpopulations to see the targeted cytotoxicity. Populations enriched for T cells by Percoll density centrifugation, treatment with anti-**CD16** plus complement or sorting for CD5+ cells, were all able to mediate **CD16** targeted cytotoxicity following activation with rIL-2. These data suggest that IL-2 activated T cells express **CD16** in addition to CD3.

23/3,AB/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07510171 91029171

Potentiation of tumor lysis by a **bispecific** **antibody** that binds to CA19-9 antigen and the **Fc** **gamma** **receptor** expressed by human large granular lymphocytes.

de Palazzo IG; Gercel-Taylor C; Kitson J; Weiner LM
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.

Cancer Res (UNITED STATES) Nov 15 1990, 50 (22) p7123-8, ISSN 0008-5472 Journal Code: CNF
Contract/Grant No.: CA06926, CA, NCI; CA01130, CA, NCI; CA50633, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Murine monoclonal antibody therapy of human cancer rarely induces clinical responses. Antibody-induced cellular infiltrates rarely accumulate at sites of tumor, even in clinically responding lesions. Thus, the ability of these antibodies to promote host effector cell-mediated lysis of tumor via antibody-dependent cellular cytotoxicity (ADCC) has not been harnessed by existing treatment approaches. One potential explanation is that ADCC requires binding of antibody Fc domains to cellular **Fc** **gamma** **receptors**, and therapeutically administered murine antibodies must compete with vast excesses of human IgG for **Fc** **gamma** **receptor** occupancy. Chemically linked antibody heteroconjugates that bind selected target and effector cell structures via distinct Fab portions can mediate lysis of malignant cells in vitro in the presence of human serum. This approach addresses a potentially major obstacle to antibody therapy.

Production of bispecific monoclonal antibodies with similar specificities and superior in vivo biodistribution characteristics would thus have potential clinical applications. We have prepared and purified a bispecific, monovalent monoclonal antibody and evaluated its in vitro effects. The IgG1-secreting hybridoma line 3G8 (alpha-human Fc gamma R III) was fused with the hybridoma line CA19-9, which produces an IgG1 antibody that binds to a glycoprotein shed by gastrointestinal cancers. Multiple clones with bispecific binding properties were identified. CA19-9 x 3G8 clonal supernatants and purified antibody, but not the parent antibodies, efficiently mediated specific in vitro lysis of cells of the SW948 line by human large granular lymphocytes (LGLs). Human serum-resistant target cell lysis augmentation at low effector:target ratios was seen using picogram amounts of antibody. In contrast, the IgG2 alpha variant of CA19-9, which also promotes ADCC by LGLs, was unable to augment lysis of SW948 cells when effectors were preincubated with human serum. This bispecific, monovalent monoclonal antibody is an efficient promoter of the anti-tumor effects of LGLs in physiological concentrations of human serum. In vivo models that evaluate treatment efficacy and promotion of inflammatory tumor infiltrates by bispecific monoclonal antibodies are required to assess the therapeutic potential of these novel constructs.

23/3,AB/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1996 Knight-Ridder Info. All rts. reserv.

07310490 90217490
Targeted cytotoxic cells in human peripheral blood lymphocytes.
Garrido MA; Perez P; Titus JA; Valdayo MJ; Winkler DF; Barbieri SA;
Wunderlich JR; Segal DM
Experimental Immunology Branch, National Cancer Institute, Bethesda, MD
20892.
J Immunol(UNITED STATES) Apr 15 1990, 144 (8) p2891-8, ISSN
0022-1767 Journal Code: IFB
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We have isolated subsets of cells from human PBL and have investigated their abilities to mediate lysis targeted by ****bispecific** **antibodies****. Targeted cytotoxic cells were divided into two distinct types based on buoyant density. The low buoyant density fraction contained all of the targetable cytotoxic activity in unstimulated PBL, including both T and K cells targeted with anti-CD3 and anti-Fc gamma RIII (****CD16****) containing ****bispecific** **antibodies****, respectively. Both types of targetable cytotoxic cells required IL-2 for maintenance of cytotoxic activity, expressed the CD56 (NKH1) marker, and mediated MHC-unrestricted lysis. The targetable T cells in low density PBL were exclusively CD8+ and represented only about 2% of the total PBL. The high buoyant density lymphocytes, depleted of NK cells, had no targetable activity, but were able to generate over several days, targetable T cell activity in the presence of a TCR cross-linking signal plus IL-2. Unlike the low-density cells, the activated high buoyant density effector T cells did not express CD56, consisted of both CD4+ and CD8+ cells, and did not mediate MHC-unrestricted lysis. These cells proliferated more rapidly and generated more total lytic activity than the low-density fraction. Our studies show that targetable cytotoxic activity in human PBL is mediated by several subsets of cells with different activation requirements. Presumably all of these activities could be directed against unwanted cells in clinical or preclinical studies involving targeted cytotoxic cells.

23/3,AB/37 (Item 37 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1996 Knight-Ridder Info. All rts. reserv.

07155028 90062028
Targeting of cytotoxic cells against tumors with heterocrosslinked, ****bispecific** **antibodies****.
Segal DM; Qian JH; Garrido MA; Perez P; Winkler DF; Wunderlich JR; Snider DP; Valdayo MJ; Titus JA
Experimental Immunology Branch, National Cancer Institute, Bethesda, Maryland 20892.
Princess Takamatsu Symp (UNITED STATES) 1988, 19 p323-31,

Journal Code: HHI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Cytotoxic cells express specific receptors on their surfaces by which they distinguish altered or foreign cells from normal autologous cells. Recently, a method has been developed by which the natural recognition system of cytotoxic cells can be artificially manipulated, giving rise to cytotoxic cells of any desired specificity, including specificity against tumor and virally infected cells. The method for retargeting cytotoxic cells employs heterocrosslinked antibodies, in which one antibody is directed against the cytotoxic cell receptor (CCR) involved in lysis, while the second antibody is directed against a target cell structure, for example a tumor or viral antigen. By linking the CCR directly to the target cell, the heterocrosslinked antibodies promote the formation of effector: target conjugates and signal the cytotoxic cell to deliver a lethal hit. T cells can be targeted by heteroconjugates containing antibodies against components of the T cell receptor complex, e.g., Ti or CD3, while several types of antibody-dependent cellular cytotoxicity (ADCC) effector cells, including K/NK cells, macrophages, and neutrophils, are targeted using heteroconjugates containing antibodies against **Fc** **gamma** **receptors**. In peripheral blood from normal donors at least six types of targetable activities have been identified in vitro. In Winn type tumor neutralization assays in nude mice, targeted T and K cells can prevent the establishment of subcutaneous tumor at low effector: tumor ratios. Moreover, targeted human peripheral blood T cells cause the eradication of established intraperitoneal human ovarian carcinoma in nude mouse models. Targeted cytotoxic cells therefore hold great promise as a novel form of cancer immunotherapy in humans.

23/3,AB/38 (Item 1 from file: 55)

DIALOG(R)File 55:BIOSIS PREVIEWS(R)

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13138282 BIOSIS Number: 99138282

In vitro tumor growth inhibition by **bispecific** **antibodies** to human transferrin receptor and tumor-associated antigens is augmented by the iron chelator deferoxamine

Hsieh-Ma S T; Shi T; Reeder J; **Ring D B**

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Clinical Immunology and Immunopathology 80 (2). 1996. 185-193.

Full Journal Title: Clinical Immunology and Immunopathology

ISSN: 0090-1229

Language: ENGLISH

Print Number: Biological Abstracts Vol. 102 Iss. 006 Ref. 086413

Previously, a panel of mouse monoclonal antibodies (mAbs) to several tumor-associated antigens was chemically crosslinked to an IgG1 anti-human transferrin receptor antibody, 454A12. We called this new class of **bispecific** **antibodies** (BmAbs) "antigen forks" and showed that these antigen forks inhibited but did not completely prevent tumor cell growth. We speculated that the conjugates acted by heterologously crosslinking two antigens in a manner that interfered with the functions of one or both. The most effective BmAbs all shared one specificity for the human transferrin receptor. A monoclonal antibody to this receptor has been shown by others to reduce tumor cell growth when used with the iron chelator deferoxamine. When we combined our antigen forks with deferoxamine, two of five BmAbs synergized with deferoxamine to arrest tumor cell count at or below input levels. The most effective BmAbs were 317G5/454A12 (3/4) and 520C9/454A12 (5/4). mAb 317G5 recognizes a 42-kDa tumor-associated glycoprotein, and mAb 520C9 recognizes the c-erbB-2 protooncogene product. BmAb 3/4 was most effective against colorectal cancer cell line HT-29, and BmAb 5/4 was most effective against breast cancer cell line SK-BR-3. When deferoxamine and BmAb were replaced by fresh medium after a 6- or 7-day treatment period, no regrowth of tumor cells was observed during the next 4 days, although regrowth was seen if either deferoxamine or BmAb was used alone. Our results show that BmAbs with specificities for transferrin receptor and certain tumor-associated antigens effectively inhibit tumor growth in vitro. When used in combination with deferoxamine, such BmAbs may have therapeutic potential for the treatment of cancer.

23/3,AB/39 (Item 2 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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12227497 BIOSIS Number: 98827497

A new in vitro model of specific targeting therapy of cancer: Retargeting of PWM-LAK cells with ****bispecific**** ****antibodies**** of greatly enhances cytotoxicity to hepatocellular carcinoma

Saijyo S; Kudo T; Katayose Y; Saeki H; Chiba N; Suzuki M; Tominaga T; Matsuno S

Cancer Cell Repository, Inst. Dev. Aging and Cancer, Tohoku Univ., 4-1 Seiryomachi, Aoba-ku, Sendai 980-77, Japan

Tohoku Journal of Experimental Medicine 178 (2). 1996. 113-127.

Full Journal Title: Tohoku Journal of Experimental Medicine

ISSN: 0040-8727

Language: ENGLISH

Print Number: Biological Abstracts Vol. 101 Iss. 012 Ref. 177919

For the purpose of establishing a new in vitro model of adoptive immunotherapy, we synthesized two kinds of ****bispecific**** ****antibodies**** (BsAbs), i.e., (OK x L) BsAbs constructed with both OKT-3 (anti-CD3) and L-7-6 (anti-HCC), and (3GxL) BsAbs constructed with 3-G-8 (anti-****CD16****) and L-7-6 antibodies. These two BsAbs, having pairs of binding arms on their single molecule, showed similar binding to target cells as the parental monoclonal antibodies (OKT-3, 3-G-8 and L-7-6), when examined with FACS. Newly devised in vitro cytotoxicity tests revealed that LAK or PWM-stimulated LAK (PWMLAK) cells did not show any significant cytotoxic activity to HCC cells, while both effector cells equally showed greatly enhanced cytotoxicity to HCC even at a low effector/target (0.3) in the presence of BsAbs (OK x L) for the efficient retargeting of the effector cells. Inasmuch as PWM-LAK cells proliferate in vitro 3-5 times faster than LAK cells, adoptive immunotherapy using PWM-LAK cells in combination with (OK x L) BsAbs should be very promising.

23/3,AB/40 (Item 3 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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12213258 BIOSIS Number: 98813258

Human targets for ****bispecific**** ****antibody****-directed therapeutics in Fc-gamma-RIII transgenic mice

Von Mehren M; Wolf E J; Amoroso A; Barth M; Franke T F; Adams G P; Ravetch J S; Weiner L M

Fox Chase Cancer Cent., Philadelphia, PA 19111, USA

Proceedings of the American Association for Cancer Research Annual Meeting 37 (0). 1996. 444.

Full Journal Title: 87th Annual Meeting of the American Association for Cancer Research, Washington, D.C., USA, April 20-24, 1996. Proceedings of the American Association for Cancer Research Annual Meeting

ISSN: 0197-016X

Language: ENGLISH

Document Type: CONFERENCE PAPER

Print Number: Biological Abstracts/RRM Vol. 048 Iss. 006 Ref. 101727

23/3,AB/41 (Item 4 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
(c) 1996 BIOSIS. All rts. reserv.

12083293 BIOSIS Number: 98683293

Binding characteristics and antitumor properties of 1A10 ****bispecific**** ****antibody**** recognizing gp40 and human transferrin receptor

Amoroso A R; Clark J I; Litwin S; Hsieh-Ma S; Shi T; Alpaugh R K; Adams G P; Wolff E J; ****Ring D B****; Weiner L M

Dep. Med. Oncol., Fox Chase Cancer Cent., 7701 Burholme Ave., Philadelphia, PA 19111, USA

Cancer Research 56 (1). 1996. 113-120.

Full Journal Title: Cancer Research

ISSN: 0008-5472

Language: ENGLISH

Print Number: Biological Abstracts Vol. 101 Iss. 006 Ref. 083574

The bispecific murine monoclonal antibody (MAb) 1A10 has specificity for

the human transferrin receptor (TfR) and the human tumor-associated antigen gp40. This antibody, therefore, functions as an "antigen fork" by binding to two distinct antigens on the same malignant cell. Highly purified 1A10 inhibits the growth of cells coexpressing high levels of human TfR and the tumor-associated antigen gp40 by binding to both target antigens. In SW948 cells, the majority of 1A10 binding is via its gp40 specificity, and half-maximal inhibition of cell growth by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay requires 2030-mu-g/ml concentrations of 1A10. The binding of 1A10 correlates with growth inhibition in the cell lines HT-29, SK-OV-3, OVCAR-2, and OVCAR-3. The growth of OVCAR-10 cells, which express little gp40 and TfR, is not inhibited by 1A10. However, SK-BR-3 cells, which express abundant gp40 and extremely high levels of TfR, are insensitive to the effects of 1A10. In some cell lines, combined exposure to 1A10 and the iron chelator deferoxamine mesylate has synergistic antiproliferative effects. A single i.p. dose of 600 mu-g 1A10 is sufficient to achieve an estimated tumor concentration of at least 30 mu-g/ml for 7 days in C.B17/lcr-scid mice bearing SW948 human tumor xenografts. Treatment of scid mice bearing day 2 or day 4 SW948 xenografts with single or multiple 1A10 doses inhibits tumor growth in a dose-related fashion. Antitumor effects are not seen with therapy using either parental antibody of 1A10. The antiproliferative properties of 1A10 in tumor cells overexpressing gp40 and TfR suggest avenues for the development of new ****bispecific** **antibody****-promoted treatment strategies.

23/3,AB/42 (Item 5 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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12022970 BIOSIS Number: 98622970
Potentiation of targeted and activated NK cells for therapy of acute myeloid leukemia with ****bispecific** **antibody**** to CD33 and ****CD16** (Fc-gamma-RIII)**
Zhong R K; Shultz L D; Chen J; Jiang B; Whiteside T L; Ball E D
Univ. Pittsburgh, Dep. Med., Pittsburgh, PA, USA
Blood 86 (10 SUPPL. 1). 1995. 789A.
Full Journal Title: 37th Annual Meeting of the American Society of Hematology, Seattle, Washington, USA, December 1-5, 1995. Blood
ISSN: 0006-4971
Language: ENGLISH
Document Type: CONFERENCE PAPER
Print Number: Biological Abstracts/RRM Vol. 048 Iss. 002 Ref. 027313

23/3,AB/43 (Item 6 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
(c) 1996 BIOSIS. All rts. reserv.

11800164 BIOSIS Number: 98400164
Killing and imaging of human neuroblastoma with an anti-G-D2-anti Fc-gamma-RI ****bispecific** **antibody****
Michon J M; Moutel S; Perdereau B; Brixy F; Barbet J; Deo Y M; Fridman W H; Teillaud J L
Lab. Biotechnol. Anticorps, INSERM U. 255, Serv. Pediatrie, Lab. Physiopathol., Inst. Curie, Paris, France
O (0). 1995. 888.
Full Journal Title: 9TH INTERNATIONAL CONGRESS OF IMMUNOLOGY. The 9th International Congress of Immunology; Meeting Sponsored by the American Association of Immunologists and the International Union of Immunological Societies, San Francisco, California, USA, July 23-29, 1995. 311p. 9th International Congress of Immunology: San Francisco, California, USA.
ISSN: *****
Language: ENGLISH
Document Type: CONFERENCE PAPER
Print Number: Biological Abstracts/RRM Vol. 047 Iss. 009 Ref. 162567

23/3,AB/44 (Item 7 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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11468842 BIOSIS Number: 98068842

Potentiation of leukemia cell lysis by a ****bispecific** antibody**** to CD33 and the Fc-gamma-RIII receptor (****CD16****) expressed by human natural killer (NK) cells

Silla L M R; Malley V; Chen J; Zhong R K; Whiteside T L; Ball E D
Div. Hematol./Bone Marrow Transplantation, Univ. Pittsburgh Med. Cent.,
Pittsburgh, PA, USA

Blood 84 (10 SUPPL. 1). 1994. 50A.

Full Journal Title: Abstracts Submitted to the 36th Annual Meeting of
the American Society of Hematology, Nashville, Tennessee, USA, December
2-6, 1994. Blood

ISSN: 0006-4971

Language: ENGLISH

Document Type: CONFERENCE PAPER

Print Number: Biological Abstracts/RRM Vol. 047 Iss. 002 Ref. 030429

23/3,AB/45 (Item 8 from file: 55)

DIALOG(R)File 55:BIOSIS PREVIEWS(R)

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11312944 BIOSIS Number: 97512944

Comparison of the ability of red cells sensitized with a bispecific
anti-D X anti-Fc-gamma-RIII Fab fragment to activate human K cells and
peritoneal macrophages through Fc-gamma-RIII

Bakacs T; Hadley A G; Kumpel B M; Segal D M; Banhidy F

Natl. Inst. Oncol., Rath Gy.u. 7-9, Budapest 1122, HUN

Immunology Letters 42 (1-2). 1994. 91-95.

Full Journal Title: Immunology Letters

ISSN: 0165-2478

Language: ENGLISH

Print Number: Biological Abstracts Vol. 098 Iss. 011 Ref. 148683

The functional activity of FcγRIII on human K cells from peripheral blood
was compared with that of FcγRIII on peritoneal macrophages (PM) separated
from the waste material of patients undergoing peritoneal dialysis.
Fc-gamma-R function was assessed in vitro using human monoclonal IgG1
anti-D (AB5) or a ****bispecific** antibody**** comprising Fab fragments of
AB5 chemically linked to Fab fragments of monoclonal antiFc-gamma-RIII, 3G8
(AB5 times 3G8). In antibody-dependent cell-mediated cytotoxicity (ADCC)
assays, K cells mediated the lysis of papainized red cells sensitized with
the AB5 times 3G8 ****bispecific** antibody**** but not with AB5. In
contrast, red cell lysis by PM was not promoted by AB5 times 3G8 although
AB5 was active. However, this lysis, being inhibited by monomeric IgG, was
presumably mediated via Fc-gamma-RI. AB5 times 3G8 also failed to promote
the binding and phagocytosis of both papainized and native red cells by PM
although 99% of red cells and over 90% of peritoneal cells bound the
****bispecific** antibody****. In marked contrast to K cells therefore,
FcγRIII on PM was unable to mediate functional interactions with red cells
sensitized with anti-D times antiFc-gamma-RIII ****bispecific** antibody****.

23/3,AB/46 (Item 9 from file: 55)

DIALOG(R)File 55:BIOSIS PREVIEWS(R)

(c) 1996 BIOSIS. All rts. reserv.

11138919 BIOSIS Number: 97338919

Improvements in hybridoma culture and ****bispecific** antibody****
production

Inlow D; Lowe D; Howarth B; MacDonald H; Harano D; Davis J; Maiorella B;
Brannon M; Fordham D; Lin L; Reeder J; ****Ring D B****

Chiron Corp., Emeryville, CA 94608, USA

Journal of Cellular Biochemistry Supplement 0 (18D). 1994. 188.

Full Journal Title: Keystone Symposium on Antibody Engineering: Research
and Application of Genes Encoding Immunoglobulins, Lake Tahoe, California,
USA, March 7-13, 1994. Journal of Cellular Biochemistry Supplement

ISSN: 0733-1959

Language: ENGLISH

Document Type: CONFERENCE PAPER

Print Number: Biological Abstracts/RRM Vol. 046 Iss. 008 Ref. 116637

23/3,AB/47 (Item 10 from file: 55)

DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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10990754 BIOSIS Number: 97190754

Targeting HIV-1 to Fc-gamma-R on human phagocytes via ****bispecific****
****antibodies**** reduces infectivity of HIV-1 to T cells

Howell A L; Guyre P M; You K-S; Panger M W
Dep. Microbiol., HB 7550, Dartmouth Medical Sch., Hanover, NH 03755-3842,
USA

Journal of Leukocyte Biology 55 (3). 1994. 385-391.

Full Journal Title: Journal of Leukocyte Biology

ISSN: 0741-5400

Language: ENGLISH

Print Number: Biological Abstracts Vol. 097 Iss. 009 Ref. 124417

In addition to CD4, the primary receptor to which the human immunodeficiency virus type 1 (HIV-1) binds, mononuclear phagocytes (monocytes) express three classes of Fc receptors for immunoglobulin G (Fc-gamma-R). We have previously shown that infection of monocytes by HIV-1 is inhibited when ****bispecific** **antibodies**** (BsAbs) are used to target the virus to either the type I, type II, or type III Fc-gamma-R on these cells. Infection of monocytes was not inhibited when HIV-1 was targeted to either human leukocyte antigen class I or CD33. We have extended these studies to examine the ability of BsAbs plus polymorphonuclear leukocytes (neutrophils, PMNs) and monocytes to reduce infectivity of HIV-1 to cells from the human T cell lymphoma line, H9. The production of HIV-1 following interaction of virus with BsAb and phagocytes was determined in an indicator cell assay by mixing BsAb, HIV-1, and phagocytes with uninfected H9 cells. Productive infection of H9 cells was quantitated on subsequent days by measuring p24 gag antigen levels in supernatants by enzyme-linked immunosorbent assay. Our findings show that the addition of interferon-gamma-activated PMNs or monocytes to cultures of HIV-1 plus H9 cells in the absence of BsAb results in a marked reduction in p24 levels equivalent to 85 to 90% of control levels. With the combination of BsAb (anti-Fc-gamma-R1 times anti-gp120) plus IFN-gamma-activated phagocytes, levels of p24 in H9 cultures were below those at culture initiation. These findings demonstrate that IFN-gamma-activated phagocytes can affect the natural course of HIV-1 infection of T cells, a finding of potential clinical importance.

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DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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10280854 BIOSIS Number: 45080854

NEUTRALIZING EFFECTS AND ADCC IN LENTIVIRAL INFECTION MEDIATED BY
****BISPECIFIC** **ANTIBODY****

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WORLD CONGRESS; MEETING, BERLIN, GERMANY, JUNE 6-11, 1993. 639P. IXTH

INTERNATIONAL CONFERENCE ON AIDS: BERLIN, GERMANY. 0 (0). 1993. 210.

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Language: ENGLISH

Document Type: CONFERENCE PAPER

23/3,AB/49 (Item 12 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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10006804 BIOSIS Number: 95006804

ANTITUMOR EFFECTS OF A ****BISPECIFIC** **ANTIBODY**** TARGETING CA19-9
ANTIGEN AND ****CD16****

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CANCER RES 52 (20). 1992. 5713-5719. CODEN: CNREA

Full Journal Title: Cancer Research

Language: ENGLISH

Bispecific murine monoclonal antibodies that target tumor and Fc.gamma.RIII (**CD16**) can promote relevant tumor lysis by large granular lymphocytes. For these antibodies to be clinically useful, their properties should be maintained in vivo, where competing human immunoglobulin, shed target antigen, and shed **CD16** may be encountered. At a minimum, **bispecific** **antibody** antitumor effects should be preserved in whole blood. Furthermore, potentiation of tumor lysis should be reflected by demonstrating the ability of **bispecific** **antibody** -retargeted effector cells to infiltrate and mediate lysis of organized tumor. If these characteristics are demonstrated, and there is evidence of in vivo efficacy of **bispecific** **antibody** -based therapy in a relevant animal model, further clinical development of such antibodies would be warranted. In this report the ability of CL158 **bispecific** **antibody** supernatants to mediate lysis of SW948 tumor growing in monolayer is shown to be preserved in the presence of interleukin 2-activated whole blood. When SW948 cells were grown in vitro as multicellular human tumor spheroids, incubation with interleukin 2-activated lymphocytes (LAK cells) and CL158 led to structural and widespread necrosis. This was dependent on CL158 and resistant to competition by pooled human immunoglobulin or interleukin 2-exposed whole blood. These effects were not promoted by the monospecific antibodies produced by the parent clone of CD158 and were not observed when the IgG2a variant of CA19-9 antibody, which mediates conventional antibody-dependent cellular cytotoxicity, was used instead of its bispecific derivative. To examine the efficacy of **bispecific** **antibody** -based treatments on in vivo tumor, scid mice bearing early s.c. SW948 xenografts were treated with interleukin 2 for 5 consecutive days, supplemented by three i.v. injections of 107 human LAK cells and various antibodies. Treatment of mice bearing SW948 tumors with LAK cells did not retard tumor growth, but when CL158 was added, significant delays in tumor growth were observed. Tumor growth delay required treatment with both LAK cells and the **bispecific** **antibody**. Treatment with the IgG2a variant of CA19-9 antibody, alone with LAK cells, had no effects on tumor growth. Although the mechanisms of these antitumor effects require further study, it is clear that human LAK cells treatment of animals bearing early, established s.c. tumors is enhanced by the addition of **bispecific** **antibodies** with relevant binding characteristics. When compared with the IgG2a isotype variant of CA19-9 monoclonal antibody, this bispecifically antibody offers the advantages of preservation of activity in physiological conditions, infiltration and disruption of organized tumor in vitro, and antitumor effects in a relevant xenograft model. These characteristics support the continued clinical development of **bispecific** **antibodies** with specificity for tumor and Fc.gamma.RIII.

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 DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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8540223 BIOSIS Number: 92005223
 FUNCTIONS OF THE VARIOUS HUMAN IGG FC RECEPTORS IN MEDIATING KILLING OF TOXOPLASMA-GONDII

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J IMMUNOL 146 (9). 1991. 3145-3151. CODEN: JOIMA

Full Journal Title: Journal of Immunology

Language: ENGLISH

The three types of IgG FcR (Fc.gamma.RI, Fc.gamma.RII, Fc.gamma.RIII) on human leukocytes play an important role in elimination of antibody-coated infectious agents. To further understand the role of the different Fc.gamma.R in mediating this killing, we examined the ability of human myeloid and lymphoid cells to kill the protozoan *Toxoplasma gondii* in the presence of antitoxoplasma IgG or **bispecific** **antibodies**. Although human myeloid cells (monocytes, macrophages, neutrophils, and eosinophils) all lysed unsensitized *T. gondii*, killing by these cells was significantly enhanced by opsonization with antitoxoplasma rabbit IgG. Human lymphocytes, however, did not lyse *T. gondii* unless the parasites were coated with antibody. The role of antibody and Fc.gamma.R in mediating ADCC of *T. gondii* was then examined using **bispecific** **antibodies** made by chemically cross-linking Fab fragments of antitoxoplasma antibodies to Fab fragments of antibodies specific for human leukocyte surface Ag, including Fc.gamma.R. Thus, simultaneous binding of these bispecifics to parasites

and effector cells allowed an evaluation of killing when *T. gondii* were targeted to each Ag independently. Bispecifics which targeted *T. gondii* to Fc.gamma.RI, II or III enhanced lysis by monocytes. However, similar results were obtained with bispecifics targeting *T. gondii* to non-Fc.gamma.R Ag (CD11b or .beta.2-microglobulin on monocytes. Likewise, polymorphonuclear leukocytes mediated significantly more lysis in the presence of bispecifics linking *T. gondii* to Fc.gamma.RII, Fc.gamma.RIII, or the two non-Fc.gamma.R Ag CD11b and .beta.2-microglobulin. Thus, although human myeloid cells did not require antibody-Fc.gamma.R triggering to kill *T. gondii*, antibody appeared to enhance lysis by capturing and directing the parasites onto the effector cell surface. Human lymphocytes, in contrast, mediated significant lysis of *T. gondii* only in the presence of bispecifics targeting *T. gondii* to Fc.gamma.RIII, indicating a requirement for specific triggering of Fc.gamma.RIII for killing by large granular lymphocytes. Consequently, using bispecifics to compare targeting to specific Ag, both non-Fc.gamma.R and Fc.gamma.R, allowed determination of the role of antibody-Fc.gamma.R, allowed determination of the role of antibody-Fc.gamma.R interactions in *T. gondii* killing. In addition, these studies demonstrate the potential of bispecifics in determining the role of specific Ag in killing of or infection by pathogens.

23/3,AB/51 (Item 14 from file: 55)
 DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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8108596 BIOSIS Number: 91029596
 POTENTIATION OF TUMOR LYSIS BY A **BISPECIFIC** **ANTIBODY** THAT BINDS
 TO CA19-9 ANTIGEN AND THE **FC**.**GAMMA** **RECEPTOR** EXPRESSED BY HUMAN
 LARGE GRANULAR LYMPHOCYTES

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CANCER RES 50 (22). 1990. 7123-7128. CODEN: CNREA

Full Journal Title: Cancer Research

Language: ENGLISH

Murine monoclonal antibody therapy of human cancer rarely induces clinical responses. Antibody-induced cellular infiltrates rarely accumulate at sites of tumor, even in clinically responding lesions. Thus, the ability of these antibodies to promote host effector cell-mediated lysis of tumor via antibody-dependent cellular cytotoxicity (ADCC) has not been harnessed by existing treatment approaches. One potential explanation is that ADCC requires binding of antibody Fc domains to cellular **Fc**.**gamma**.**receptors**, and therapeutically administered murine antibodies must compete with vast excesses of human IgG for **Fc**.**gamma**.**receptor** occupancy. Chemically linked antibody heteroconjugates that bind selected target and effector cell structures via distinct Fab portions can mediate lysis of malignant cells in vitro in the presence of human serum. This approach addresses a potentially major obstacle to antibody therapy. Production of bispecific monoclonal antibodies with similar specificities and superior in vivo biodistribution characteristics would thus have potential clinical applications. We have prepared and purified a bispecific, monovalent monoclonal antibody and evaluated its in vitro effects. The IgG1-secreting hybridoma line 3G8 (.alpha.-human Fc.gamma.R III) was fused with the hybridoma line CA19-9, which produces an IgG1 antibody that binds to a glycoprotein shed by gastrointestinal cancers. Multiple clones with bispecific binding properties were identified. CA19-9.times. 3G8 clonal supernatants and purified antibody, but not the parent antibodies, efficiently mediated specific in vitro lysis of cells of the SW948 line by human large granular lymphocytes (LGLs). Human serum-resistant target cell lysis augmentation at low effector:target ratios was seen using picogram amounts of antibody. In contrast, the IgG2.alpha. variant of CA19-9, which also promotes ADCC by LGLs, was unable to augment lysis of SW948 cells when effectors were preincubated with human serum. This bispecific, monovalent monoclonal antibody is an efficient promoter of the anti-tumor effects of LGLs in physiological concentrations of human serum. In vivo models that evaluate treatment efficacy and promotion of inflammatory tumor infiltrates by bispecific monoclonal antibodies are required to access the therapeutic potential of these novel constructs.

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DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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7743496 BIOSIS Number: 90111496

EVALUATION OF THE ANTIBODY-DEPENDENT CYTOTOXIC CAPABILITIES OF INDIVIDUAL HUMAN MONOCYTES ROLE OF FC-GAMMA-RI AND FC-GAMMA-RII AND THE EFFECTS OF CYTOKINES AT THE SINGLE CELL LEVEL

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J IMMUNOL 145 (5). 1990. 1483-1489. CODEN: JOIMA
Full Journal Title: Journal of Immunology
Language: ENGLISH

In this report we present evidence that not all human peripheral blood monocytes mediate antibody-dependent cellular cytotoxicity (ADCC), and that this function may be determined on an individual cell by both the type and level of expression of FcR, and by the state of cellular activation and/or differentiation. Although the diverse range of effector and regulatory functions performed by human monocytes suggests the possibility of distinct subsets, it is not clear whether observed functional heterogeneity reflects the presence of true monocyte subpopulations, or whether this diversity represents a continuum of maturational states present in the peripheral circulation. In an attempt to address this question, we investigated the ability of human monocytes to carry out ADCC at the single cell levels, with emphasis on the role of the three FcR for IgG (Fc.gamma.RI, Fc.gamma.RII, and Fc.gamma.RIII) in mediating cytotoxicity. Using a modified plaque assay, 58.3% +/- 4.9 of freshly isolated monocytes mediated ADCC, as evidenced by the formation for lytic plaques in monolayers of ox erythrocyte (oxE) target cells. Significant increases in the number of plaque-forming cells were observed after positive selection by flow microfluorimetry for those monocytes expressing high levels of Fc.gamma.RI and Fc.gamma.RII, but not Fc.gamma.RIII. ****Bispecific** antibodies**** composed of Fab fragments of anti-oxE antibody covalently coupled to Fab fragments of anti-Fc.gamma.R antibodies were used to independently evaluate the ability of Fc.gamma.RI, Fc.gamma.RII, and Fc.gamma.RIII to mediate single cell cytotoxicity. Significant increases in the number of plaque-forming cells were observed in the presence of anti-Fc.gamma.RI .times. anti-oxE and anti-Fc.gamma.RII .times. anti-oxE ****bispecific** antibodies****, confirming the efficiency of Fc.gamma.RI and Fc.gamma.RII as cytotoxic trigger molecules on human monocytes. Incubation of monocytes with purified rIFN-.gamma. and granulocyte macrophage-CSF, but not IL-2, IL-3, IL-4, IL-6, or TNF-.alpha., also resulted in significant increases in the number of monocytes mediating cytotoxicity, suggesting that cytotoxic ability at the single cell level may be influenced by factors which effect monocyte activation and differentiation, respectively. Overall, these studies demonstrate that freshly isolated human monocytes are heterogeneous in their ability to mediate ADCC, and suggest that this functional diversity arises not from discrete subpopulations of cells, but from a continuum of maturation/activational states present within the peripheral circulation.

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DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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7328223 BIOSIS Number: 38108744

HYBRID HYBRIDOMA PRODUCING ****BISPECIFIC** ANTIBODIES**** TO HUMAN BREAST CANCER ASSOCIATED ANTIGENS AND HUMAN FC RECEPTOR III

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XIVTH INTERNATIONAL MEETING OF THE SOCIETY FOR ANALYTICAL CYTOLOGY,
ASHEVILLE, NORTH CAROLINA, USA, MARCH 18-23, 1990. CYTOMETRY 0 (SUPPL. 4).
1990. 72. CODEN: CYTOD
Language: ENGLISH
Document Type: CONFERENCE PAPER

23/3,AB/54 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 1996 Elsevier Science B.V. All rts. reserv.

10070959 EMBASE No: 96247175

Treatment of Hodgkin's disease with ****bispecific**** ****antibodies****
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Annals of Oncology (Netherlands) , 1996, 7/SUPPL. 4 (S143-S146) CODEN:
ANONE ISSN: 0923-7534

LANGUAGES: English SUMMARY LANGUAGES: English

Bispecific monoclonal antibodies (Bi-MAbs) with dual specificity for tumor-associated antigens (TAA) and a triggering molecule of an immunologic effector cell, respectively, open the possibility to specifically target to and activate cytotoxic effector cells (macrophages, T-cells, NK cells) at the tumor site. Using appropriately designed Bi-MAbs and unstimulated human NK cells and T-cells, respectively, we were able to cure SCID mice xenografted with human Hodgkin's tumors. This approach was also effective in disseminated tumors and when treatment was delayed until three weeks after the inoculation of the tumor, thus establishing this approach as the most effective model of an immunomodulating therapy of human neoplasms. Early observations with an ongoing phase I/II study with ****CD16****/CD30 Bi-MAb in patients with refractory Hodgkin's disease confirm the expected low toxicity. If these observations can be confirmed in larger clinical studies, effector cell activating Bi-MAbs could become an important weapon in the remaining fight for the conquest of Hodgkin's disease.

23/3,AB/55 (Item 2 from file: 73)

DIALOG(R)File 73:EMBASE

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9776470 EMBASE No: 95336581

Treatment of heterotransplanted hodgekin's tumors in SCID mice by a combination of human NK or T cells and ****bispecific**** ****antibodies****
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Journal of Hematotherapy (USA) , 1995, 4/5 (447-451) CODEN: JOEME
ISSN: 1061-6128

LANGUAGES: English SUMMARY LANGUAGES: English

To test the feasibility and efficacy of a new immunotherapeutic approach in Hodgkin's disease, bispecific monoclonal antibodies (BsmAb) were established with specificity for the Hodgkin's-associated CD30 antigen and for ****CD16**** (on NK cells) or CD3 and CD28 (on T lymphocytes), respectively. These BsmAb induced a specific and efficient NK cell or T cell-mediated cytotoxicity in vitro. The treatment of severe combined immunodeficiency (SCID) mice with the NK (anti-****CD16****/CD30) or T cell (anti-CD3/CD30 and anti- CD28/CD30) activating BsmAb followed by administration of resting human lymphocytes led to complete remission of established heterotransplanted human Hodgkin's tumors. Even disseminated tumors were cured. Studies on the mechanism responsible for tumor destruction revealed that treatment efficacy depended on lymphocyte activation at the tumor site. Localization of human lymphocytes in mice was BsmAb mediated and antigen specific as activated lymphocytes were only detected in CD30+ tumors but not in CD30- colorectal carcinomas coestablished as a control in the same animal.

23/3,AB/56 (Item 3 from file: 73)

DIALOG(R)File 73:EMBASE

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8941627 EMBASE No: 93245347

****Bispecific**** ****antibody**** as a potentiator of tumor cell killing by IL-2- activated lymphocytes

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GANN MONOGR. CANCER RES. (Japan) , 1993, 40/- (95-106) CODEN: GANMA
ISSN: 0072-0151

LANGUAGES: English SUMMARY LANGUAGES: English

Lymphokine-activated killer (LAK) cells, which can be readily induced from peripheral blood lymphocytes (PBL) by culturing with interleukin 2 (IL-2) and exhibit broad cytotoxicity against various tumor target cells,

have been the subject of clinical trials of adoptive immunotherapy of cancer patients. Such trials throughout the world, however, have resulted in limited success to date. This appears to result largely from the inefficient recognition of various target cells by LAK cells, the mechanisms for which are still unclear. In order to bypass such an obscure step in the LAK cell-mediated cytotoxicity, we used heteroconjugated antibodies composed of a monoclonal antibody (mAb) to triggering molecules on LAK cells, such as CD3 and **CD16**, chemically cross-linked with a mAb to a glioma-associated antigen. Such **bispecific** **antibodies** (BsAb) redirected the LAK cell cytotoxicity against glioma cells in a highly specific and effective manner in vitro. The specific targeting therapy using LAK cells in conjunction with BsAb resulted in a striking clinical efficacy in post-operational treatment of glioma patients as compared with the classical LAK therapy.

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 DIALOG(R)File 73:EMBASE
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8715620 EMBASE No: 93019382

A human tumor xenograft model of therapy with a bispecific monoclonal antibody targeting **c-erbB-2** and **CD16**

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CANCER RES. (USA), 1993, 53/1 (94-100) CODEN: CNREA ISSN: 0008-5472
 LANGUAGES: English SUMMARY LANGUAGES: English

New strategies are required to clinically exploit the ability of monoclonal antibodies to target tumor for lysis by cellular effector mechanisms. In this report we examine the therapeutic effects of 2B1, a bispecific monoclonal antibody with specificity for the extracellular domain of the **c-erbB-2** oncogene product and the human Fc gamma receptor, Fc gammaRIII (**CD16**), describe the characteristics and limitations of this model, and examine the mechanisms underlying the observed responses. The model uses SK-OV-3 human ovarian carcinoma xenografts in scid mice. These cells are susceptible to 2B1-directed lysis by human peripheral blood lymphocytes or lymphokine-activated killer cells, and maintain **c-erbB-2** expression in vivo. 125I-labeled 2B1 selectively accumulates in tumor, with a peak of 10.5% injected dose/g of tumor 24 h following its i.v. injection. However, the selectivity of this binding is lessened by 2B1 accumulation in the lungs and other normal organs and persistence in the blood. This is caused by antibody binding to murine lung, colon, stomach, and skin expressing the epitope recognized by the anti-**c-erbB-2** component of 2B1 in tumor-bearing, but not normal mice. In treatment studies using various permutations of antibody, human peripheral blood lymphocytes or lymphokine-activated killer cells and interleukin 2, cellular therapy alone had minimal effects on SK-OV-3 xenograft growth, but significantly improved when 2B1 treatment was incorporated. Median survivals increased from 80 plus or minus 3.5 days with no therapy to 131 plus or minus 7.3 days following therapy with 100 microg 2B1, interleukin 2, and human peripheral blood lymphocytes, with 70% of animals exhibiting no evidence of tumor at day 150. These effects were preserved when the cells were administered in human serum. In contrast, human serum abolished the antitumor effects of 520C9, which is the parent anti-**c-erbB-2** antibody of 2B1. Thus 2B1-based therapy has therapeutic effects, without obvious toxicity, despite the targeting of this antibody to normal murine tissues. Since combinations of 2B1 and interleukin 2 may have antitumor properties, mechanisms other than bispecific monoclonal antibody-promoted conjugation of **c-erbB-2** antigen-expressing tumor to **CD16**-expressing effector cells may be involved.

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8680307 EMBASE No: 92358954

Targeting of T or NK lymphocytes against tumor cells by bispecific monoclonal antibodies: Role of different triggering molecules

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INT. J. CANCER (USA), 1992, -/SUPPL. 7 (15-18) CODEN: IJCNA ISSN:
0020-7136 ADONIS ORDER NUMBER: 002071369200477H

LANGUAGES: English SUMMARY LANGUAGES: English

MAbs directed against triggering surface molecules expressed by T lymphocytes (CD3, TCR, CD2, CD28) or by NK cells (CD2, **CD16**) are able to induce the functional program of these cells. These MAbs represent suitable reagents to construct biMAbs directed against TAA, in order to specifically target effector lymphocytes against tumor cells. Anti-CD3/anti-EGF-R biMAbs were constructed to specifically direct T lymphocytes against EGF-R+ tumor cells. Such biMAbs are able to induce cytotoxicity of EGF-R+ tumor cell lines (A431, IGROV, KATO-III and U-87) by cytotoxic CD3+ effector lymphocytes while tumor cells having low or absent expression of EGF-R were not lysed. In addition, both cytotoxic T (CD8+) cells and non-cytotoxic (CD4+) IL-2-expanded lymphocytes were able to secrete lymphokines upon contact with EGF-R+ tumor cells. To target NK cells against NK resistant ovarian carcinomas, we used an anti-**CD16** MAb (IgG1) together with an anti-ovarian carcinoma MAb (IgG(2a)), to construct biMAbs using the hybrid hybridoma technique. The hybrid IgG1/IgG(2a) biMAb triggered the specific lysis of relevant target cells by resting NK cells and by a subset of NK clones. In addition, some TCR gamma/delta+ clones but not TCR alpha/beta+ clones could be targeted by the biMAb.

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DIALOG(R)File 73:EMBASE

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8141500 EMBASE No: 91172118

Bispecific monoclonal antibodies directed to **CD16** and to a tumor-associated antigen induce target-cell lysis by resting NK cells and by a subset of NK clones

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INT. J. CANCER (USA), 1991, 48/2 (227-233) CODEN: IJCNA ISSN:
0020-7136 ADONIS ORDER NUMBER: 0020713691002328

LANGUAGES: English

CD16 surface antigens represent activating molecules in CD3-16+ NK cells. In order to target NK cells against relatively NK-resistant ovarian carcinomas, we used an anti-**CD16** monoclonal antibody (MAb) (VD4), together with an anti-ovarian carcinoma-associated antigen (MOV19), to construct biMAbs. To this end, hybrid hybridomas were generated by fusing a TK-deficient VD4 hybridoma mutant with a HGPRT-deficient MOV19 hybrid. Supernatants from hybrid hybridomas that had been selected in HAT medium were screened for their ability to induce a CD3-16+ NK clone to lyse an MOV19+ ovarian carcinoma cell line in a 4-hr ⁵¹Cr-release assay. The NMB.45 hybrid hybridoma secreted a biMAb which triggered lysis of MOV19+ but not of MOV19- target cells. Some degree of target cell lysis was also observed with MOV19 MAb (due to ADCC mechanisms), while the VD4 MAb was ineffective. HPLC fractionation of MAbs secreted by the hybrid hybridoma made it possible to identify 4 different peaks, one of which appeared to contain functional biMAb molecules. HPLC-purified biMAb (100 ng/ml) induced resting PBL to lyse the 'NK-resistant' IGROV1 ovarian carcinoma cell line. Fresh MOV19+ tumor cells were also lysed, although with lower efficiency. When IL-2-activated lymphocytes were used as a source of effectors, biMAb caused only minor increases in the IL-2-induced cytotoxic activity. Further analyses of the effect of biMAb were performed at the clonal level. Among CD3-16+ NK cell clones, a clear enhancing effect could be observed only in GL183+ but not in GL183- clones. In CD3+ cytotoxic clones a triggering effect could be detected in one out of 4 TCR gamma/beta+ clones but not in TCR alpha/beta+ clones.

?Es29/3,ab/all

29/3,AB/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09700678 96222278

Human neutrophil interactions of a bispecific monoclonal **antibody** targeting tumor and human Fc gamma RIII.

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Cancer Immunol Immunother (GERMANY) Mar 1996, 42 (3) p141-50, ISSN 0340-7004 Journal Code: CN3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

****2B1**** is a bispecific murine monoclonal **antibody** (bsmAb) targeting the ****c**.-**erbB****-2 and CD16 (Fc gamma RIII) antigens. ****c**.-**erbB****-2 is over-expressed by a variety of adenocarcinomas, and CD16, the low-affinity Fc gamma receptor for aggregated immunoglobulins, is expressed by polymorphonuclear leukocytes (PMN), natural killer (NK) cells and differentiated mononuclear phagocytes. ****2B1**** potentiates the in vitro lysis of c-erb-2 over-expressing tumors by NK cells and macrophages. In this report, the interactions between ****2B1**** and PMN were investigated to assess the impact of these associations on in vitro ****2B1****-promoted tumor cytotoxicity by human NK cells. The peak binding of ****2B1**** to PMN was observed at a concentration of 10 microgram/ml ****2B1****. However, ****2B1**** rapidly dissociated from PMN in vitro at 37 degrees C in non-equilibrium conditions. This dissociation was not caused by CD16 shedding. When PMN were labeled with ¹²⁵I-****2B1**** and incubated at 37 degrees C and the supernatants examined by HPLC analysis, the Fab regions of dissociated ****2B1**** were not complexed with shed CD16 extracellular domain. While most of the binding of ****2B1**** PMN was solely attributable to Fab-directed binding to Fc gamma RIII, PMN-associated ****2B1**** also bound through Fc gamma-domain/Fc gamma RII interactions. ****2B1**** did not promote in vitro PMN cytotoxicity against ****c**.-**erbB****-2-expressing SK-OV-3 tumor cells. When PMN were coincubated with peripheral blood lymphocytes, SK-OV-3 tumor and ****2B1****, the concentration of ****2B1**** required for maximal tumor lysis was lowered. Although PMN may serve as a significant competitive binding pool of systemically administered ****2B1**** in vivo, the therapeutic potential of the targeted cytotoxicity properties of this bsmAb should not be compromised.

29/3,AB/2 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09607882 96129482

Clinical development of ****2B1****, a bispecific murine monoclonal **antibody** targeting ****c**.-**erbB****-2 and Fc gamma RIII.

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J Hematother (UNITED STATES) Oct 1995, 4 (5) p453-6, ISSN 1061-6128
Journal Code: B3T

Contract/Grant No.: CM27732-49, CM, NCI; CA50633, CA, NCI; CA58262, CA, NCI; +

Languages: ENGLISH

Document type: CLINICAL TRIAL; CLINICAL TRIAL, PHASE I; JOURNAL ARTICLE

Bispecific monoclonal **antibodies** (BsmAb) can be used to specifically target tumor cells for cytotoxicity mediated by defined effector cells. One such BsmAb, ****2B1****, targets the extracellular domains of both the ****c**.-**erbB****-2 protein product of the ****HER****-****2****/neu oncogene and Fc gamma RIII (CD16), the Fc gamma receptor expressed by human natural killer cells, neutrophils, and differentiated mononuclear phagocytes. ****2B1**** promotes the conjugation of cells expressing these target antigens. It efficiently promotes the specific lysis of tumor cells expressing ****c**.-**erbB****-2 by human NK cells and macrophages over a broad concentration range. ****2B1**** selectively targets ****c**.-**erbB****-2-positive human tumor xenografts growing in immunodeficient SCID mice. Treatment of such mice with ****2B1**** plus interleukin 2 (IL-2) inhibits the growth of early, established human tumor xenografts overexpressing ****c**.-**erbB****-2. A phase I clinical trial of ****2B1**** has been initiated to determine the toxicity profile and maximum tolerated dose (MTD) of this BsmAb and to examine the biodistribution of the **antibody** and the biologic effects of treatment. Preliminary results of this trial indicate that the dose-limiting toxicity for patients with

extensive prior bone marrow-toxic therapy is thrombocytopenia for as yet undetermined reasons. Toxicities of fevers, rigors, and associated constitutional symptoms are explained, in part, by treatment-induced systemic expression of cytokines, such as tumor necrosis factor-alpha. Circulating, functional BsmAb is easily detectable in treatment patients' sera and exhibits complex elimination patterns. HAMA and anti-idiotypic treatment-induced **antibodies** are induced by **2B1** treatment. Some preliminary indications of clinical activity have been observed. BsmAb therapy targeting tumor antigens and Fc gamma RIII has potent immunologic effects. Future studies will include the development of more relevant animal models for BsmAb therapy targeting human Fc gamma RIII. The ongoing phase I trial will be completed to identify the MTD for patients without extensive prior bone marrow-toxic chemotherapy and radiation. A phase II clinical trial of **2B1** therapy in women with metastatic breast cancer is planned, as is a phase I trial incorporating treatment with both **2B1** and IL-2.

29/3,AB/3 (Item 3 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09607876 96129476

G-CSF-stimulated PMN in immunotherapy of breast cancer with a bispecific **antibody** to Fc gamma RI and to **HER**-**2**/neu (MDX-210).

Repp R; Valerius T; Wieland G; Becker W; Steininger H; Deo Y; Helm G; Gramatzki M; Van de Winkel JG; Lang N; et al

Department of Medicine III, University of Erlangen-Nurnberg, Germany.

J Hematother (UNITED STATES) Oct 1995, 4 (5) p415-21, ISSN 1061-6128

Journal Code: B3T

Languages: ENGLISH

Document type: CLINICAL TRIAL; CLINICAL TRIAL, PHASE I; JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL

Myeloid cells can mediate tumor cell cytotoxicity via certain receptors for immunoglobulins. Among the different Fc receptors, the high-affinity IgG receptor (Fc gamma RI, CD64) is a promising trigger molecule because it is selectively expressed on effector cells, including monocytes/macrophages and granulocyte colony-stimulating factor (G-CSF)-primed neutrophils. In vitro, a bispecific **antibody** (BsAb) (MDX-210, constructed by chemically cross-linking F(ab') fragments of monoclonal **antibody** (mAb) **520C9** to **HER**-**2**/neu and F(ab') fragments of mAb 22 to Fc gamma RI) mediated effective lysis of **HER**-**2**/neu overexpressing breast cancer cell lines. **HER**-**2**/neu (**c**-**erbB2**) is overexpressed in approximately 30% of breast and ovarian carcinomas and is a target for immunotherapy in clinical trials. In vitro assays showed Fc gamma RI-positive neutrophils to constitute a major effector cell population during G-CSF therapy. Based on these preclinical data and a preceding study at Dartmouth (New Hampshire) with a single dose of MDX-210 alone, a combination of G-CSF and MDX-210 is tested in a phase I study in breast cancer patients. In this study, patients receiving G-CSF are treated with escalating single doses of MDX-210. This therapy was generally well tolerated by the treated patients, some of whom reacted with fever and short periods of chills, which were temporally related to elevated plasma levels of IL-6 and TNF-alpha. After MDX-210 application, a transient decrease in the total white blood count and absolute neutrophil count (ANC) was observed. During G-CSF application, isolated neutrophils were highly cytotoxic in the presence of MDX-210 in vitro. These data indicate a potential role for G-CSF and BsAb in immunotherapy.

29/3,AB/4 (Item 4 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09580062 96101662

Enhanced tumor specificity of **741F8**-1 (sFv)₂, an anti-**c**-**erbB**-2 single-chain Fv dimer, mediated by stable radioiodine conjugation.

Adams GP; McCartney JE; Wolf EJ; Eisenberg J; Huston JS; Bookman MA; Moldofsky P; Stafford WF 3rd; Houston LL; Weiner LM

Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania.

J Nucl Med (UNITED STATES) Dec 1995, 36 (12) p2276-81, ISSN 0161-5505

Journal Code: JEC

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The goal of this study was to determine if the stabilization of the radioiodine-protein bond by the N-succinimidyl p-iodobenzoate (PIB) method improved the degree and specificity of tumor localization of 125I-****741F8****-1 (sFv)₂, an anti-****c****-****erbB****-2 sFv dimer, in an immunodeficient murine model. METHODS: Gamma camera images were acquired 21 hr after intravenous administration of 131I-****741F8****-1 (sFv)₂ labeled by the p-iodobenzoate or chloramine T methods. The stability of the radioiodine-protein bond also was assessed in plasma samples after intravenous injection of 125I-****741F8****-1 (sFv)₂ labeled by either the chloramine T or p-iodobenzoate methods. RESULTS: By 6 hr postinjection, 97% of the activity associated with the 125I-****741F8****-1 (sFv)₂ labeled by the p-iodobenzoate method was protein bound compared with 61% after labeling with the chloramine-T method. These observations indicate that increasing the stability of the conjugation between the radioiodine and the sFv molecule can significantly increase the degree and specificity of tumor targeting. Significantly greater tumor retention ($p < 0.005$) and lower blood ($p < 0.001$), spleen ($p < 0.001$) and stomach ($p < 0.005$) retention were observed in biodistribution studies when the p-iodobenzoate conjugate was used. This resulted in superior tumor-to-organ ratios for all tissue samples studied. CONCLUSION: These observations may have clinical relevance for the use of radiolabeled sFv as imaging agents.

29/3,AB/5 (Item 5 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09572324 96093924

Engineering disulfide-linked single-chain Fv dimers [(sFv)₂] with improved solution and targeting properties: anti-digoxin 26-10 (sFv)₂ and anti-****c****-****erbB****-2 ****741F8**** (sFv)₂ made by protein folding and bonded through C-terminal cysteinyl peptides.

McCartney JE; Tai MS; Hudziak RM; Adams GP; Weiner LM; Jin D; Stafford WF 3rd; Liu S; Bookman MA; Laminet AA; et al

Creative BioMolecules Inc., Hopkinton, MA 01748, USA.

Protein Eng (ENGLAND) Mar 1995, 8 (3) p301-14, ISSN 0269-2139

Journal Code: PR1

Contract/Grant No.: U01 CA51880, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Single-chain Fv fusions with C-terminal cysteinyl peptides (sFv) have been engineered using model sFv proteins based upon the 26-10 anti-digoxin IgG and ****741F8**** anti-****c****-****erbB****-2 IgG monoclonal ****antibodies****. As part of the ****741F8**** sFv construction process, the PCR-amplified ****741F8**** VH gene was modified in an effort to correct possible primer-induced errors. Genetic replacement of the N-terminal beta-strand sequence of ****741F8**** VH with that from the FR1 of anti-****c****-****erbB****-2 ****520C9**** VH resulted in a dramatic improvement of sFv folding yields. Folding in urea-glutathione redox buffers produced active sFv' with a protected C-terminal sulfhydryl, presumably as the mixed disulfide with glutathione. Disulfide-bonded (sFv)₂ homodimers were made by disulfide interchange or oxidation after reductive elimination of the blocking group. Both 26-10 (sFv)₂ and ****741F8**** (sFv)₂ existed as stable dimers that were well behaved in solution, whereas ****741F8**** sFv and sFv' exhibited considerable self-association. The ****741F8**** sFv binds to the extracellular domain (ECD) of the ****c****-****erbB****-2 oncogene protein, which is often overexpressed in breast cancer and other adenocarcinomas. The recombinant ECD was prepared to facilitate the analysis of ****741F8**** binding site properties; the cloned ECD gene, modified to encode a C-terminal Ser-Gly-His6 peptide, was transfected into Chinese hamster ovary cells using a vector that also expressed dihydrofolate reductase to facilitate methotrexate amplification. Optimized cell lines expressed ECD-His6 at high levels in a cell bioreactor; after isolation by immobilized metal affinity chromatography, final ECD yields were as high as 47 mg/l. An animal tumor model complemented physicochemical studies of ****741F8**** species and indicated increased tumor localization of the targeted ****741F8**** (sFv)₂ over other monovalent ****741F8**** species.

29/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09553835 96075435

Targeting **cc-erbB-2** expressing tumors using single-chain Fv monomers and dimers.
Tai MS; McCartney JE; Adams GP; Jin D; Hudziak RM; Oppermann H; Laminet AA; Bookman MA; Wolf EJ; Liu S; et al
Creative BioMolecules, Inc., Hopkinton, Massachusetts 01748, USA.
Cancer Res (UNITED STATES) Dec 1 1995, 55 (23 Suppl) p5983s-5989s,
ISSN 0008-5472 Journal Code: CNF
Contract/Grant No.: U01 CA51880, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Single-chain Fv proteins containing a COOH-terminal cysteine (sFv') were constructed by using an antidigoxin 26.10 sFv and an anti-**cc-erbB-2** **741F8** sFv. The fully active sFv' proteins were prepared by expression in Escherichia coli as insoluble inclusion bodies, followed by in vitro refolding using glutathione redox buffers and purification. The COOH-terminal cysteines of the refolded sFv' proteins were protected by a blocking group presumed to be the glutathionyl peptide, which was easily and selectively removed by gentle reduction. Air oxidation of the reduced sFv' monomers resulted in the efficient formation of disulfide-linked sFv' homodimers, designated (sFv')₂, which were stable under oxidizing conditions and relatively slow to be disrupted under reducing conditions. The (26-10-1 sFv)-(b741F8-1 sFv) heterodimer was prepared and possessed dual-antigen specificity; the active bispecific (sFv')₂ dimerized under native conditions, apparently as a manifestation of self-association by the **741F8** sFv' subunit. Biodistribution and imaging studies that were performed on mice bearing human SK-OV-3 tumor xenografts that express the **cc-erbB-2** as a cell surface antigen were reviewed. Radioiodinated **741F8-2** (sFv')₂ homodimer localized to the tumors with high specificity, as evidenced by excellent tumor:normal tissue ratios. Sagittal section autoradiography of whole animals 24 h after administration of **antibody** species revealed that **741F8** (sFv')₂ produced a stronger tumor image than comparable doses of the **741F8** Fab, monomeric sFv', and the 26-10 (sFv')₂ control without the high nonspecific background distribution of the **741F8** IgG.

29/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09484605 96006205

Phase I trial of **2B1**, a bispecific monoclonal **antibody** targeting **cc-erbB-2** and Fc gamma RIII.
Weiner LM; Clark JI; Davey M; Li WS; Garcia de Palazzo I; Ring DB; Alpaugh RK
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA.
Cancer Res (UNITED STATES) Oct 15 1995, 55 (20) p4586-93, ISSN 0008-5472 Journal Code: CNF
Contract/Grant No.: 1 R01 CA50633, CA, NCI; CM-27732-49, CM, NCI; 1 R01 CA58262, CA, NCI
Languages: ENGLISH
Document type: CLINICAL TRIAL; CLINICAL TRIAL, PHASE I; JOURNAL ARTICLE
2B1 is a bispecific murine monoclonal **antibody** (BsMAb) with specificity for the **cc-erbB-2** and Fc gamma RIII extracellular domains. This BsMAb promotes the targeted lysis of malignant cells overexpressing the **cc-erbB-2** gene product of the **HER2/neu** proto-oncogene by human natural killer cells and mononuclear phagocytes expressing the Fc gamma RIII A isoform. In a Phase I clinical trial of **2B1**, 15 patients with **cc-erbB-2**-overexpressing tumors were treated with 1 h i.v. infusions of **2B1** on days 1, 4, 5, 6, 7, and 8 of a single course of treatment. Three patients were treated with daily doses of 1.0 mg/m², while six patients each were treated with 2.5 mg/m² and 5.0 mg/m², respectively. The principal non-dose-limiting transient toxicities were fevers, rigors, nausea, vomiting, and leukopenia. Thrombocytopenia was dose limiting at the 5.0 mg/m² dose level in two patients who had received extensive prior myelosuppressive chemotherapy. Murine **antibody** was

detectable in serum following **2B1** administration, and its bispecific binding properties were retained. The pharmacokinetics of this murine **antibody** were variable and best described by nonlinear kinetics with an average $t_{1/2}$ of 20 h. Murine **antibody** bound extensively to all neutrophils and to a proportion of monocytes and lymphocytes. The initial **2B1** treatment induced more than 100-fold increases in circulating levels of tumor necrosis factor- α , interleukin 6, and interleukin 8 and lesser rises in granulocyte-monocyte colony-stimulating factor and IFN- γ . Brisk human anti-mouse **antibody** responses were induced in 14 of 15 patients. Several minor clinical responses were observed, with reductions in the thickness of chest wall disease in one patient with disseminated breast cancer. Resolution of pleural effusions and ascites, respectively, were noted in two patients with metastatic colon cancer, and one of two liver metastases resolved in a patient with metastatic colon cancer. Treatment with **2B1** BsMAb has potent immunological consequences. The maximum tolerated dose and Phase II daily dose for patients with extensive prior myelosuppressive chemotherapy was 2.5 mg/m². Continued dose escalation is required to identify the maximally tolerated dose for patients who have been less heavily pretreated.

29/3,AB/8 (Item 8 from file: 155)

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09466796 95396796

Radiometal labeling of recombinant proteins by a genetically engineered minimal chelation site: technetium-99m coordination by single-chain Fv **antibody** fusion proteins through a C-terminal cysteinyl peptide.

George AJ; Jamar F; Tai MS; Heelan BT; Adams GP; McCartney JE; Houston LL; Weiner LM; Oppermann H; Peters AM; et al

Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom.

Proc Natl Acad Sci U S A (UNITED STATES) Aug 29 1995, 92 (18) p3358-62

, ISSN 0027-8424 Journal Code: PV3

Contract/Grant No.: U01 CA51880, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We describe a method to facilitate radioimaging with technetium-99m (99mTc) by genetic incorporation of a 99mTc chelation site in recombinant single-chain Fv (sFv) **antibody** proteins. This method relies on fusion of the sFv C terminus with a Gly4Cys peptide that specifically coordinates 99mTc. By using analogues of the 26-10 anti-digoxin sFv as our primary model, we find that addition of the chelate peptide, to form 26-10-1 sFv', does not alter the antigen-binding affinity of sFv. We have demonstrated nearly quantitative chelation of 0.5-50 mCi of 99mTc per mg of 26-10-1 sFv' (1 Ci = 37 GBq). These 99mTc-labeled sFv' complexes are highly stable to challenge with saline buffers, plasma, or diethylenetriaminepentaacetic acid. We find that the 99mTc-labeled **741F8**-1 sFv', specific for the **c**-**erbB** -2 tumor-associated antigen, is effective in imaging human ovarian carcinoma in a scid mouse tumor xenograft model. This fusion chelate methodology should be applicable to diagnostic imaging with 99mTc and radioimmunotherapy with 186Re or 188Re, and its use could extend beyond the sFv' to other engineered **antibodies**, recombinant proteins, and synthetic peptides.

29/3,AB/9 (Item 9 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09393948 95323948

Optimization of in vivo tumor targeting in SCID mice with divalent forms of **741F8** anti-**c**-**erbB** -2 single-chain Fv: effects of dose escalation and repeated i.v. administration.

Adams GP; McCartney JE; Wolf EJ; Eisenberg J; Tai MS; Huston JS; Stafford WF 3rd; Bookman MA; Houston LL; Weiner LM

Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA.

Cancer Immunol Immunother (GERMANY) May 1995, 40 (5) p299-306, ISSN 0340-7004 Journal Code: CN3

Contract/Grant No.: U01 CA51880, CA, NCI; CA06927, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Single-chain Fv molecules in monovalent (sFv) and divalent [(sFv)₂] forms exhibit highly specific tumor targeting in mice as a result of their small size and rapid systemic clearance. As a consequence, there is a rapid reversal of the sFv blood/tumor gradient, resulting in diminished retention of sFv species in tumors. In this report we investigate two distinct strategies, dose escalation and repetitive intravenous (i.v.) dosing, aiming to increase the absolute selective retention of radiolabeled anti-**cc-erbB-2** 125I-**741F8** (sFv)₂ in **cc-erbB-2**-overexpressing SK-OV-3 tumors in mice with severe combined immunodeficiency (SCID). A dose-escalation strategy was applied to single i.v. injections of 125I-**741F8** (sFv)₂. Doses from 50 micrograms to 1000 micrograms were administered without a significant decrease in tumor targeting or specificity. High doses resulted in large increases in the absolute retention of 125I-**741F8** (sFv)₂. For example, raising the administered dose from 50 micrograms to 1000 micrograms increased the tumor retention 24 h after injection from 0.46 microgram/g to 9.5 micrograms/g, and resulted in a net increase of greater than 9 micrograms/g. Over the same dose range, the liver retention rose from 0.06 microgram/g to 1 microgram/g, and resulted in a net increase of less than 1 microgram/g. The retention of 9.5 micrograms/g in tumor 24 h following the 1000-micrograms dose of (sFv)₂ was comparable to that seen 24 h after a 50-micrograms dose of 125I-**741F8** IgG, indicating that the use of large doses of (sFv)₂ may partially offset their rapid clearance. When two doses were administered by i.v. injection 24 h apart, the specificity of delivery to tumor observed after the first dose was maintained following the second injection. Tumor retention of 125I-**741F8** (sFv)₂ was 0.32 microgram/g at 24 h and 0.22 micrograms/g at 48 h following a single injection of 20 micrograms, while 0.04 microgram/ml and 0.03 microgram/ml were retained in blood at the same assay times. After a second 20-micrograms injection at the 24-h assay time, tumor retention increased to 0.49 micrograms/g, and blood retention was 0.06 microgram/ml, at the 48-h point. These results suggest that multiple high-dose administrations of radiolabeled **741F8** (sFv)₂ may lead to the selective tumor localization of therapeutic radiation doses.

29/3,AB/10 (Item 10 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09324582 95254582

Tumor targeting in a murine tumor xenograft model with the (sFv)₂ divalent form of anti-**cc-erbB-2** single-chain Fv.

Huston JS; Adams GP; McCartney JE; Tai MS; Hudziak RM; Oppermann H; Stafford WF 3rd; Liu S; Fand I; Apell G; et al

Creative BioMolecules, Inc., Hopkinton, MA 01748.

Cell Biophys (UNITED STATES) 1994, 24-25 p267-78, ISSN 0163-4992

Journal Code: CQC

Contract/Grant No.: CA51880, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

This investigation has utilized novel forms of the single-chain Fv (sFv), wherein a cysteine-containing peptide has been fused to the sFv carboxyl terminus to facilitate disulfide bonding or specific cross-linking of this sFv' to make divalent (sFv)₂. The **741F8** anti-**cc-erbB-2** monoclonal **antibody** was used as the basis for construction of **741F8** sFv, from which the sFv' and (sFv)₂ derivatives were prepared. Recombinant **cc-erbB-2** extracellular domain (ECD) was prepared in CHO cells and the bivalency of **741F8** (sFv)₂ demonstrated by its complex formation with ECD. The tumor binding properties of 125I-labeled anti-**cc-erbB-2** **741F8** sFv, sFv', and (sFv)₂ were compared with radiolabeled antidigoxin 26-10 sFv' and (sFv)₂ controls. Following intravenous administration of radiolabeled species to severe combined immune-deficient (SCID) mice bearing SK-OV-3 tumors (which over-express **cc-erbB-2**), blood and organ samples were obtained as a function of time over 24 h. Comparative analysis of biodistribution and tumor-to-organ ratios demonstrated the **741F8** sFv, sFv', and (sFv)₂ had excellent specificity for tumors, which improved with time after injection. This contrasted with nonspecific interstitial pooling in tumors observed with the 26-10 sFv, sFv', and (sFv)₂, which decreased with time after administration. Tumor localization was significantly better for disulfide or peptide crosslinked

****741F8**** (sFv)² having Gly4Cys tails than for monovalent ****741F8**** sFv or Fab. The superior properties of the ****741F8**** (sFv)² in targeting SK-OV-3 tumors in SCID mice suggests the importance of further investigations of divalent sFv analogs for immunotargeting.

29/3,AB/11 (Item 11 from file: 155)
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09046794 94361794

Mammalian cell expression of single-chain Fv (sFv) ****antibody**** proteins and their C-terminal fusions with interleukin-2 and other effector domains.

Dorai H; McCartney JE; Hudziak RM; Tai MS; Laminet AA; Houston LL; Huston JS; Oppermann H

Creative BioMolecules, Inc., Hopkinton, MA 01748.

Biotechnology (N Y) (UNITED STATES) Sep 1994, 12 (9) p890-7, ISSN 0733-222X Journal Code: AL1

Contract/Grant No.: U01 CA51880, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The production of several single-chain Fv (sFv) ****antibody**** proteins was examined by three modes of mammalian cell expression. Our primary model was the ****741F8**** anti-****c****-****erbB****-2 sFv, assembled as either the VH-VL or VL-VH, and expressed alone, with C-terminal cysteine for dimerization, or as fusion proteins with carboxyl-terminal effector domains, including interleukin-2, the B domain of staphylococcal protein A, the S-peptide of ribonuclease S, or hexa-histidine metal chelate peptide. Constructs were expressed and secreted transiently in 293 cells and stably in CHO or Sp2/O cell lines, the latter yielding up to 10 mg per liter. Single-chain constructs of MOPC 315 myeloma and 26-10 monoclonal ****antibodies**** were also expressed, as were hybrids comprising unrelated VH and VL regions. Our results suggest that mammalian expression is a practical and valuable complement to the bacterial expression of single-chain ****antibodies****.

29/3,AB/12 (Item 12 from file: 155)
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09005116 94320116

Antigen forks: bispecific reagents that inhibit cell growth by binding selected pairs of tumor antigens.

Ring DB; Hsieh-Ma ST; Shi T; Reeder J

Department of Immunotherapeutics, Chiron Corporation, Emeryville, CA 94608.

Cancer Immunol Immunother (GERMANY) Jul 1994, 39 (1) p41-8, ISSN 0340-7004 Journal Code: CN3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Bispecific ****antibodies**** of a new category, termed "antigen forks", were constructed by crosslinking ****antibodies**** that recognized pairs of distinct tumor cell surface antigens. At concentrations of 1-100 nM, several such forks inhibited the growth of human tumor cell lines bearing both relevant antigens. The same cells were not inhibited by unconjugated component ****antibodies****, and the active conjugates did not inhibit the growth of human cell lines that expressed lower levels of relevant antigens. The three most active antigen forks all contained monoclonal ****antibody**** 454A12, which recognizes human transferrin receptor. This ****antibody**** was conjugated respectively to ****antibodies**** ****113F1**** (against a tumor-associated glycoprotein complex), 317G5 (against a 42-kDa tumor-associated glycoprotein), or ****520C9**** (against the ****c****-****erbB****-2 protooncogene product). The 317G5-454A12 fork strongly inhibited the HT-29 and SW948 human colorectal cancer cell lines, while the ****113F1****-454A12 and ****520C9****-454A12 forks strongly inhibited the SK-BR-3 human breast cancer cell line and the ****113F1****-454A12 fork was also effective against SW948. By designing forks against antigens of incompatible function that are co-expressed at high levels on tumor cells but not on normal tissues, it may be possible to generate reagents that inhibit tumor growth with enhanced selectivity.

29/3,AB/13 (Item 13 from file: 155)
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08900186 94215186

Gamma-interferon inhibits Fc receptor II-mediated phagocytosis of tumor cells by human macrophages.

Backman KA; Guyre PM

Department of Medicine, East Carolina University School of Medicine, Greenville, North Carolina 27858-4354.

Cancer Res (UNITED STATES) May 1 1994, 54 (9) p2456-61, ISSN 0008-5472 Journal Code: CNF

Contract/Grant No.: 1 F32 CA 08955, CA, NCI; 1-RO1-DK33100, DK, NIDDK

Languages: ENGLISH

Document type: JOURNAL ARTICLE

In vitro, monocyte-derived macrophages (MDM) are capable of efficient ****antibody****-mediated phagocytosis of human nucleated tumor cells. These MDM express on their cell surface all three classes of Fc receptors for IgG (Fc gamma R). Fc gamma R specificity for murine ****antibody**** isotype allowed us to examine the phagocytic role of Fc gamma RII on control and gamma-interferon (IFN-gamma)-primed MDM. Monoclonal ****antibody**** ****520C9**** (IgG1) mediates phagocytosis through Fc gamma RII. This monoclonal ****antibody**** is directed against the ****HER**** ****2**** /neu protooncogene product overexpressed on a variety of adenocarcinomas including the breast carcinoma cell line SK-BR-3. Our results showed that IFN-gamma treatment of differentiated MDM (days 8-12 in culture) inhibited Fc gamma RII-mediated phagocytosis in a dose-dependent manner with negative effects noted at doses as low as 0.1 units/ml. The percentage reduction in ****antibody****-mediated phagocytosis observed following IFN-gamma priming (40 units/ml for 18 h) ranged from 23-89% of control. The inhibitory effect was evident when exposure to IFN-gamma was transient. Fc gamma RII expression was not altered by IFN-gamma treatment. In our model, IFN-gamma did not up-regulate or down-regulate ****HER**** ****2**** /neu protein expression on our targets or affect the level of CD14 antigen expression on our MDM. Although IFN-gamma is a potent activator of monocytes/macrophages and can enhance certain tumoricidal mechanisms, our data show that ****antibody****-dependent phagocytosis through the type II Fc receptor is inhibited by IFN-gamma priming. Nonspecific phagocytosis was not affected.

29/3,AB/14 (Item 14 from file: 155)
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08871659 94186659

Immunohistochemical detection of ****c**** ****erbB**** -2 expression by neoplastic human tissue using monospecific and bispecific monoclonal ****antibodies****.

Garcia de Palazzo I; Klein-Szanto A; Weiner LM

Fox Chase Cancer Center, Philadelphia, PA 19111.

Int J Biol Markers (ITALY) Oct-Dec 1993, 8 (4) p233-9, ISSN 0393-6155

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Languages: ENGLISH

Document type: JOURNAL ARTICLE

Selected murine monoclonal ****antibodies**** (MAb) have been shown to inhibit relevant tumor growth in vitro and in animal models. Recently, bispecific ****antibodies**** (BsMAb) have been developed which target cytolytic effector cells via one ****antibody**** binding site and tumor antigen by the other specificity. For example, the BsMAb ****2B1**** possesses specificity for ****c**** ****erbB**** -2 and Fc gamma RIII, the low affinity Fc gamma receptor expressed by polymorphonuclear leukocytes (PMN), macrophages and large granular lymphocytes (LGL). The human homologue of the rat neu oncogene, ****c**** ****erbB**** -2, has been demonstrated to be amplified in breast, gastrointestinal, lung and ovarian carcinomas. Tumor expression of ****c**** ****erbB**** -2 has been shown to be an important prognostic indicator in breast and ovarian carcinomas. The restricted expression of the ****c**** ****erbB**** -2 protooncogene product in normal human tissues and the wide distribution of ****c**** ****erbB**** -2 expression in such tumors may justify attempts to use an appropriately constructed BsMAb in clinical trials. In this report we have addressed this issue by immunohistochemically evaluating the expression of ****c**** ****erbB**** -2 oncogene product in a variety

of malignant tumors utilizing **2B1** and the anti-**c-erbB**-2 monovalent parent of **2B1**, **520C9**. Among the studied neoplasms, **c-erbB**-2 expression was detected in 49% of primary carcinomas stained with **520C9** and in 39% of those stained with **2B1**. In the group of metastatic tumors, **c-erbB**-2 oncoprotein was detected in 52% of cases by **520C9** and in 41% by **2B1**. (ABSTRACT TRUNCATED AT 250 WORDS)

29/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08657248 93367248

Binding and cytotoxicity characteristics of the bispecific murine monoclonal **antibody** **2B1**.
Weiner LM; Holmes M; Richeson A; Godwin A; Adams GP; Hsieh-Ma ST; Ring DB ; Alpaugh RK
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111.

J Immunol (UNITED STATES) Sep 1 1993, 151 (5) p2877-86, ISSN 0022-1767 Journal Code: IFB

Contract/Grant No.: CA06927, CA, NCI; CA50633, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Bispecific monoclonal **antibodies** (BsmAb) with specificity for tumor Ag and effector cell trigger molecules have been shown to redirect the cytotoxicity of several peripheral blood mononuclear cell populations against relevant tumor. The BsmAb, **2B1**, binds to the extracellular domain of the **c-erbB**-2 gene product of the **HER2**/**neu** proto-oncogene and to CD16. In this report, the binding and cytotoxic characteristics of **2B1** are presented. Maximal saturation binding of **2B1** to PBL and **c-erbB**-2 expressing SK-OV-3 cells occurred in the 1 microgram/ml concentration range. However, substantial lysis potentiation was observed at 1000-fold lower BsmAb concentrations. Optimal tumor lysis was obtained when the BsmAb, PBL, and target cells were continuously coincubated. When PBL were franked with **2B1**, washed, and added to labeled targets, substantially less lysis was observed. These results suggest that the best way to therapeutically exploit the cytotoxic attributes of **2B1** may be to obtain continuous BsmAb exposure to tumor. Approaches based on franking of this BsmAb to PBL may not be warranted.

29/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08654902 93364902

Highly specific in vivo tumor targeting by monovalent and divalent forms of **741F8** anti-**c-erbB**-2 single-chain Fv.
Adams GP; McCartney JE; Tai MS; Oppermann H; Huston JS; Stafford WF 3d; Bookman MA; Fand I; Houston LL; Weiner LM
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.

Cancer Res (UNITED STATES) Sep 1 1993, 53 (17) p4026-34, ISSN 0008-5472 Journal Code: CNF

Contract/Grant No.: U01 CA51880, CA, NCI; CA06927, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The in vivo properties of monovalent and divalent single-chain Fv (sFv)-based molecules with the specificity of the anti-**c-erbB**-2 monoclonal **antibody** **741F8** were examined in scid mice bearing SK-OV-3 tumor xenografts. **741F8** sFv monomers exhibited rapid, biphasic clearance from blood, while a slightly slower clearance was observed with the divalent **741F8** (sFv)₂ comprising a pair of **741F8** sFv with a C-terminal Gly4Cys joined by a disulfide bond. Following i.v. injection, the **741F8** sFv monomer was selectively retained in **c-erbB**-2-overexpressing SK-OV-3 tumor, with excellent tumor:normal organ ratios uniformly exceeding 10:1 by 24 h. The specificity of this effect was demonstrated by the lack of retention of the anti-digoxin 26-10 sFv monomer, as evaluated by biodistribution studies, gamma camera imaging, and cryomacroautoradiography studies. The specificity index (**741F8** sFv retention/26-10 sFv retention) of **741F8** monomer binding, measured by

the percentage of injected dose per g of tissue, was 13.2:1 for tumor, and 0.8 to 2.1 for all tested normal organs, with specificity indices for tumor:organ ratios ranging from 7.0 (kidneys) to 16.7 (intestines). Comparing divalent **741F8** (sFv)₂ with the 26-10 (sFv)₂, similar patterns emerged, with specificity indices for retention in tumor of 16.9 for the Gly4Cys-linked (sFv)₂. These data demonstrate that, following their i.v. administration, both monovalent and divalent forms of **741F8** sFv are specifically retained by SK-OV-3 tumors. This antigen-specific binding, in conjunction with the 26-10 sFv controls, precludes the possibility that passive diffusion and pooling in the tumor interstitium contributes significantly to long-term tumor localization. **741F8** (sFv)₂ species with peptide spacers exhibited divalent binding and increased retention in tumors as compared with **741F8** sFv monomers. Since the blood retention of the (sFv)₂ is slightly more prolonged than that of the monomer, it was necessary to demonstrate that the increased tumor localization of the peptide-linked (sFv)₂ was due to its divalent nature. The significantly greater localization of the divalent bismalimidohexane-linked **741F8** (sFv)₂ as compared with a monovalent **741F8** Fab fragment of approximately the same size suggests that the increased avidity of the (sFv)₂ is a factor in its improved tumor retention. This is the first report of successful specific in vivo targeting of tumors by divalent forms of sFv molecules. The improved retention of specific divalent (sFv)₂ by tumors may have important consequences for targeted diagnostic or therapeutic strategies.

29/3,AB/17 (Item 17 from file: 155)
 DIALOG(R)File 155:MEDLINE(R)
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08395424 93105424

A human tumor xenograft model of therapy with a bispecific monoclonal **antibody** targeting **c**.*erbB**₂ and CD16.

Weiner LM; Holmes M; Adams GP; LaCreta F; Watts P; Garcia de Palazzo I
 Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.

Cancer Res (UNITED STATES) Jan 1 1993; 53 (1) p94-100, ISSN 0008-5472
 Journal Code: CNF

Contract/Grant No.: CA50633, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

New strategies are required to clinically exploit the ability of monoclonal **antibodies** to target tumor for lysis by cellular effector mechanisms. In this report we examine the therapeutic effects of **2B1**₂, a bispecific monoclonal **antibody** with specificity for the extracellular domain of the **c**.*erbB**₂ oncogene product and the human Fc gamma receptor, Fc gamma RIII (CD16), describe the characteristics and limitations of this model, and examine the mechanisms underlying the observed responses. The model uses SK-OV-3 human ovarian carcinoma xenografts in scid mice. These cells are susceptible to **2B1**₂-directed lysis by human peripheral blood lymphocytes or lymphokine-activated killer cells, and maintain **c**.*erbB**₂ expression in vivo. ¹²⁵I-labeled **2B1**₂ selectively accumulates in tumor, with a peak of 10.5% injected dose/g of tumor 24 h following its i.v. injection. However, the selectivity of this binding is lessened by **2B1**₂ accumulation in the lungs and other normal organs and persistence in the blood. This is caused by **antibody** binding to murine lung, colon, stomach, and skin expressing the epitope recognized by the anti-**c**.*erbB**₂ component of **2B1**₂ in tumor-bearing, but not normal mice. In treatment studies using various permutations of **antibody**₂, human peripheral blood lymphocytes or lymphokine-activated killer cells and interleukin 2, cellular therapy alone had minimal effects on SK-OV-3 xenograft growth, but significantly improved when **2B1**₂ treatment was incorporated. Median survivals increased from 80 +/- 3.5 days with no therapy to 131 +/- 7.3 days following therapy with 100 micrograms **2B1**₂, interleukin 2, and human peripheral blood lymphocytes, with 70% of animals exhibiting no evidence of tumor at day 150. These effects were preserved when the cells were administered in human serum. In contrast, human serum abolished the antitumor effects of **520C9**₂, which is the parent anti-**c**.*erbB**₂ **antibody** of **2B1**₂. Thus **2B1**₂-based therapy has therapeutic effects, without obvious toxicity, despite the targeting of this **antibody**₂ to normal murine tissues. Since combinations of **2B1**₂ and interleukin 2 may have antitumor properties,

mechanisms other than bispecific monoclonal **antibody**-promoted conjugation of **c-erbB-2** antigen-expressing tumor to CD16-expressing effector cells may be involved.

29/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08382128 93092128

In vitro cytotoxic targeting by human mononuclear cells and bispecific **antibody** **2B1**, recognizing **c-erbB-2** protooncogene product and Fc gamma receptor III.

Hsieh-Ma ST; Eaton AM; Shi T; Ring DB
Chiron Corporation, Emeryville, California 94608.
Cancer Res (UNITED STATES) Dec 15 1992, 52 (24) p6832-9, ISSN 0008-5472 Journal Code: CNF

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Bispecific murine monoclonal **antibody** **2B1**, possessing dual specificity for the human **c-erbB-2** protooncogene product and human Fc gamma receptor III (CD16) was evaluated for the ability to promote specific lysis of **c-erbB-2**-positive tumor cells in vitro. In short-term 51Cr release assays with human mononuclear cells as effectors and SK-Br-3 human breast cancer cells as targets, neither parental **antibody** of **2B1** mediated significant specific lysis, but bispecific **antibody** was as active as a chemical heteroconjugate, with 5 ng/ml of **2B1** causing half-maximal lysis at an effector/target ratio of 20:1 and 2 ng/ml **2B1** causing half-maximal lysis at an E/T ratio of 40:1. The cytotoxic targeting activity of **2B1** F(ab)2 fragment was the same as that of whole bispecific **antibody**, and the activity of whole **2B1** was not reduced when assays were performed in 100% autologous human serum, indicating that **2B1** binds effector cells through the CD16-binding site derived from parental **antibody** 3G8 rather than through its Fc portion. Variable inhibition of **2B1**-mediated lysis was observed when autologous polymorphonuclear leukocytes from different donors were added to mononuclear effector cells at a 2:1 ratio; this inhibition was overcome at higher **antibody** concentration. **2B1** bispecific monoclonal **antibody** was also able to mediate targeted cytolysis using whole human blood as a source of effector cells or using effector or target cells derived from ovarian cancer patients.

29/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08169960 92307960

A combination of two immunotoxins exerts synergistic cytotoxic activity against human breast-cancer cell lines.

Crews JR; Maier LA; Yu YH; Hester S; O'Brian K; Leslie DS; DeSombre K; George SL; Boyer CM; Argon Y; et al
Department of Medicine, Duke University Medical Center, Durham, NC 27710.
Int J Cancer (UNITED STATES) Jul 9 1992, 51 (5) p772-9, ISSN 0020-7136 Journal Code: GQU

Contract/Grant No.: 5-R01-CA39930, CA, NCI; P01 CA47741, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

In previous studies, combinations of immunotoxins reactive with different cell-surface antigens have exerted additive cytotoxicity against tumor cells in culture. In this report we describe a combination of 2 immunotoxins that produce synergistic cytotoxic activity. Recombinantly derived ricin A chain (RTA) was conjugated with murine monoclonal **antibodies** (MAbs) 317G5, 26OF9, 454A12 and **741F8** that bound to cell-surface determinants of 42, 55, 180 (transferrin receptor) and 185 kDa (**HER-2/neu**) expressed by the SKBr3 human breast-cancer cell line. When inhibition of clonogenic growth was measured in a limiting dilution assay, the combination of 26OF9-RTA and 454A12-RTA produced synergistic cytotoxic activity against SKBr3 and 2 other breast-cancer cell lines. All other combinations produced only additive inhibition of clonogenic growth. Simultaneous binding of 26OF9 and 454A12 was not supra-additive, but sub-populations of cells which lacked one or the other antigen could be

detected. Kinetic studies of internalization, using **antibodies** conjugated with gold particles, indicated that 454A12 remained within peripheral endosomes for a longer interval in the presence of 260F9. This change in the traffic of the transferrin receptor may contribute to synergy between 260F9-RTA and 454A12-RTA.

29/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07830601 91349601

Selection of hybrid hybridomas by flow cytometry using a new combination of fluorescent vital stains.

Shi T; Eaton AM; Ring DB
Department of Immunology, Cetus Corporation, Emeryville, CA 94608.
J Immunol Methods (NETHERLANDS) Aug 9 1991, 141 (2) p165-75, ISSN 0022-1759 Journal Code: IFE

Languages: ENGLISH
Document type: JOURNAL ARTICLE

A new combination of fluorescent dyes (rhodamine 123 and hydroethidine) was used to internally label hybridoma fusion partners. Murine hybridoma **520C9** (recognizing human **c**.*erbB** -2) was labeled with hydroethidine. Murine hybridoma 3G8 (recognizing human Fc gamma receptor III) was labeled with rhodamine 123, and verapamil was used to block rhodamine efflux via P-glycoprotein. Viability assays showed little cytotoxicity from these dyes at the concentrations used. The labeled cells were fused with polyethylene glycol, sorted for dual fluorescence on an Epics V cell sorter, and cloned. Hybrid hybridomas producing bispecific **antibodies** were selected for ability to promote lysis of SK-Br-3 breast cancer cells by human mononuclear cells. Several positive clones were obtained and shown to have a double content of DNA. Bispecific **antibody** produced by subclone **2B1** was purified by anion exchange chromatography and shown to bind both tumor cells and Fc gamma R III bearing cells. Using two parameter flow cytometric analysis, we were able to measure a 'bridging' effect of this bispecific **antibody**, which caused formation of complexes between PMNs and SK-Br-3 cells. Either parental **antibody** could compete with bispecific **antibody** to block such complexing. This fusion method provides several advantages over other techniques presently used (speed, convenience, low toxicity and automatic exclusion of dead cells) and can be applied to produce other hybrid hybridomas.

29/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07823699 91342699

Identity of BCA200 and **c**.*erbB** -2 indicated by reactivity of monoclonal **antibodies** with recombinant **c**.*erbB** -2 [published erratum appears in Mol Immunol 1992 Feb;29(2):291-2]

Ring DB; Clark R; Saxena A
Department of Immunology, Cetus Corporation, Emeryville, CA 94068.
Mol Immunol (ENGLAND) Aug 1991, 28 (8) p915-7, ISSN 0161-5890
Journal Code: NG1

Languages: ENGLISH
Document type: JOURNAL ARTICLE

BCA200 has been described as a 200,000 Mr monomeric cell surface glycoprotein associated with human breast cancer. Since the physical properties and cellular distribution of BCA200 resemble those of **c**.*erbB** -2, **antibodies** to BCA200 were tested for the ability to bind a recombinant protein containing the **c**.*erbB** -2 extracellular domain (erbB-2 ECD). Three **antibodies** to distinct epitopes of BCA200 reacted with erbB-2 ECD but not with a control protein expressed in a similar baculovirus lysate. Control myeloma proteins and **antibodies** to four other antigens did not react with erbB-2 ECD. A protein with the expected molecular weight for erbB-2 ECD was also immunoprecipitated by anti-BCA200 **antibody** **520C9**. We conclude that BCA200 is another synonym for **c**.*erbB** -2.

29/3,AB/22 (Item 1 from file: 55)

DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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13138282 BIOSIS Number: 99138282

In vitro tumor growth inhibition by bispecific ****antibodies**** to human transferrin receptor and tumor-associated antigens is augmented by the iron chelator deferoxamine

Hsieh-Ma S T; Shi T; Reeder J; Ring D B

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Clinical Immunology and Immunopathology 80 (2). 1996. 185-193.

Full Journal Title: Clinical Immunology and Immunopathology

ISSN: 0090-1229

Language: ENGLISH

Print Number: Biological Abstracts Vol. 102 Iss. 006 Ref. 086413

Previously, a panel of mouse monoclonal ****antibodies**** (mAbs) to several tumor-associated antigens was chemically crosslinked to an IgG1 anti-human transferrin receptor ****antibody****, 454A12. We called this new class of bispecific ****antibodies**** (BmAbs) "antigen forks" and showed that these antigen forks inhibited but did not completely prevent tumor cell growth. We speculated that the conjugates acted by heterologously crosslinking two antigens in a manner that interfered with the functions of one or both. The most effective BmAbs all shared one specificity for the human transferrin receptor. A monoclonal ****antibody**** to this receptor has been shown by others to reduce tumor cell growth when used with the iron chelator deferoxamine. When we combined our antigen forks with deferoxamine, two of five BmAbs synergized with deferoxamine to arrest tumor cell count at or below input levels. The most effective BmAbs were 317G5/454A12 (3/4) and ****520C9****/454A12 (5/4). mAb 317G5 recognizes a 42-kDa tumor-associated glycoprotein, and mAb ****520C9**** recognizes the ****c**-*erbB****-2 protooncogene product. BmAb 3/4 was most effective against colorectal cancer cell line HT-29, and BmAb 5/4 was most effective against breast cancer cell line SK-BR-3. When deferoxamine and BmAb were replaced by fresh medium after a 6- or 7-day treatment period, no regrowth of tumor cells was observed during the next 4 days, although regrowth was seen if either deferoxamine or BmAb was used alone. Our results show that BmAbs with specificities for transferrin receptor and certain tumor-associated antigens effectively inhibit tumor growth in vitro. When used in combination with deferoxamine, such BmAbs may have therapeutic potential for the treatment of cancer.

29/3,AB/23 (Item 2 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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13042030 BIOSIS Number: 99042030

Human neutrophil interactions of a bispecific monoclonal ****antibody**** targeting tumor and human Fc-gamma-RIII

Weiner L M; Alpaugh R K; Amoroso A R; Adams G P; Ring D B; Barth M W
Dep. Med. Oncol., Fox Chase Cancer Cent., 7701 Burholme Ave., Philadelphia, PA 19111, USA

Cancer Immunology Immunotherapy 42 (3). 1996. 141-150.

Full Journal Title: Cancer Immunology Immunotherapy

ISSN: 0340-7004

Language: ENGLISH

Print Number: Biological Abstracts Vol. 102 Iss. 002 Ref. 024203

****2B1**** is a bispecific murine monoclonal ****antibody**** (bsmAb) targeting the ****c**-*erbB****-2 and CD16 (Fc-gamma-RIII) antigens. ****c**-*erbB****-2 is over-expressed by a variety of adenocarcinomas, and CD16, the low-affinity Fc-gamma receptor for aggregated immunoglobulins, is expressed by polymorphonuclear leukocytes (PMN), natural killer (NK) cells and differentiated mononuclear phagocytes. ****2B1**** potentiates the in vitro lysis of ****c**-*erbB****-2 over-expressing tumors by NK cells and macrophages. In this report, the interactions between ****2B1**** and PMN were investigated to assess the impact of these associations on in vitro ****2B1****-promoted tumor cytotoxicity by human NK cells. The peak binding of ****2B1**** to PMN was observed at a concentration of 10 μ -g/ml ****2B1****. However, ****2B1**** rapidly dissociated from PMN in vitro at 37 degree C in non-equilibrium conditions. This dissociation was not caused by CD16 shedding. When PMN were labeled with 125I-****2B1**** and incubated at 37 degree C and the supernatants examined by HPLC analysis, the Fab regions of

dissociated **2B1** were not complexed with shed CD16 extracellular domain. While most of the binding of **2B1** to PMN was solely attributable to Fab-directed binding to Fc-gamma-RIII, PMN-associated **2B1** also bound through Fc-gamma-domain/Fc-gamma-RII interactions. **2B1** did not promote in vitro PMN cytotoxicity against **c**-**erbB**-2-expressing SK-OV-3 tumor cells. When PMN were coincubated with peripheral blood lymphocytes, SK-OV-3 tumor and **2B1**, the concentration of **2B1** required for maximal tumor lysis was lowered. Although PMN may serve as a significant competitive binding pool of systemically administered **2B1** in vivo, the therapeutic potential of the targeted cytotoxicity properties of this bsmAb should not be compromised.

29/3,AB/24 (Item 3 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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12110876 BIOSIS Number: 98710876
Therapeutic efficacy of doxorubicin immunoconjugates targeting the
HER-**2**-neu oncogenic protein in a human breast cancer cell line
Specht J S; Sivam G P; Gralow J R
Div. Oncol., Univ. Wash., Seattle, WA, USA
Journal of Investigative Medicine 44 (1). 1996. 136A.
Full Journal Title: Meeting of the American Federation for Clinical
Research, Western Region, Carmel, California, USA, February 14-17, 1996.
Journal of Investigative Medicine
ISSN: 1081-5589
Language: ENGLISH
Document Type: CONFERENCE PAPER
Print Number: Biological Abstracts/RRM Vol. 048 Iss. 004 Ref. 057058

29/3,AB/25 (Item 4 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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11996889 BIOSIS Number: 98596889
Targeting **c**-**erbB**-2 expressing tumors using single-chain FV
monomers and dimers
Tai M-S; McCartney J E; Adams G P; Jin D; Hudziak R M; Oppermann H;
Lamiet A A; Bookman M A; Wolf E J; Liu S; Stafford W F III; Fand I;
Houston L L; Weiner L M; Huston J S
Creative BioMol. Inc., 45 South St., Hopkinton, MA 01748, USA
Cancer Research 55 (23 SUPPL.). 1995. 5983S-5989S.
Full Journal Title: Cancer Research
ISSN: 0008-5472
Language: ENGLISH
Print Number: Biological Abstracts Vol. 101 Iss. 002 Ref. 024594
Single-chain Fv proteins containing a COOH-terminal cysteine (sFv) were
constructed by using an antidigoxin 26-10 sFv and an anti-**c**-**erbB**-2
741 F8 sFv. The fully active sFv proteins were prepared by expression in
Escherichia coli as insoluble inclusion bodies, followed by in vitro
refolding using glutathione redox buffers and purification. The
COOH-terminal cysteines of the refolded sFv proteins were protected by a
blocking group presumed to be the glutathionyl peptide, which was easily
and selectively removed by gentle reduction. Air oxidation of the reduced
sFv monomers resulted in the efficient formation of disulfide-linked sFv
homodimers, designated (sFv)-2, which were stable under oxidizing
conditions and relatively slow to be disrupted under reducing conditions.
The (26-10-1 sFv)-(**741F8**-1 sFv) heterodimer was prepared and
possessed dual-antigen specificity; the active bispecific (sFv)-2
dimerized under native conditions, apparently as a manifestation of
self-association by the **741F8** sFv subunit. Biodistribution and imaging
studies that were performed on mice bearing human SK-OV-3 tumor xenografts
that express the **c**-**erbB**-2 as a cell surface antigen were reviewed.
Radioiodinated **741F8**-2 (sFv)-2 homodimer localized to the tumors with
high specificity, as evidenced by excellent tumor:normal tissue ratios.
Sagittal section autoradiography of whole animals 24 h after administration
of **antibody** species revealed that **741F8** (sFv)-2 produced a
stronger tumor image than comparable doses of the **741F8** Fab, monomeric
sFv, and the 26-10 (sFv)-2 control without the high nonspecific
background distribution of the **741F8** IgG.

29/3,AB/26 (Item 5 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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11950958 BIOSIS Number: 98550958

Phase I trial of ****2B1****, a bispecific monoclonal ****antibody**** targeting ****c*-erbB*-2** and Fc-gamma-RIII

Weiner L M; Clark J I; Davey M; Li W S; De Palazzo I G; Ring D B; Alpaugh R K

Dep. Med. Oncol., Fox Chase Cancer Cent., 7701 Burholme Ave,
Philadelphia, PA 19111, USA

Cancer Research 55 (20). 1995. 4586-4593.

Full Journal Title: Cancer Research

ISSN: 0008-5472

Language: ENGLISH

Print Number: Biological Abstracts Vol. 100 Iss. 012 Ref. 187649

****2B1**** is a bispecific murine monoclonal ****antibody**** (BsMAb) with specificity for the ****c*-erbB*-2** and Fc-gamma-RIII extracellular domains. This BsMAb promotes the targeted lysis of malignant cells overexpressing the ****c*-erbB*-2** gene product of the ****HER2****/neu proto-oncogene by human natural killer cells and mononuclear phagocytes expressing the Fc-gamma-RIII A isoform. In a Phase I clinical trial of ****2B1****, 15 patients with ****c*-erbB*-2**-overexpressing tumors were treated with 1 h i.v. infusions of ****2B1**** on days 1, 4, 5, 6, 7, and 8 of a single course of treatment. Three patients were treated with daily doses of 1.0 mg/m², while six patients each were treated with 2.5 mg/m² and 5.0 mg/m², respectively. The principal non-dose-limiting transient toxicities were fevers, rigors, nausea, vomiting, and leukopenia. Thrombocytopenia was dose limiting at the 5.0 mg/m² dose level in two patients who had received extensive prior myelosuppressive chemotherapy. Murine ****antibody**** was detectable in serum following ****2B1**** administration, and its bispecific binding properties were retained. The pharmacokinetics of this murine ****antibody**** were variable and best described by nonlinear kinetics with an average t-1/2 of 20 h. Murine ****antibody**** bound extensively to all neutrophils and to a proportion of monocytes and lymphocytes. The initial ****2B1**** treatment induced more than 100-fold increases in circulating levels of tumor necrosis factor-alpha, interleukin 6, and interleukin 8 and lesser rises in granulocyte-monocyte colony-stimulating factor and IFN-gamma. Brisk human anti-mouse ****antibody**** responses were induced in 14 of 15 patients. Several minor clinical responses were observed, with reductions in the thickness of chest wall disease in one patient with disseminated breast cancer. Resolution of pleural effusions and ascites, respectively, were noted in two patients with metastatic colon cancer, and one of two liver metastases resolved in a patient with metastatic colon cancer. Treatment with ****2B1**** BsMAb has potent immunological consequences. The maximum tolerated dose and Phase II daily dose for patients with extensive prior myelosuppressive chemotherapy was 2.5 mg/m². Continued dose escalation is required to identify the maximally tolerated dose for patients who have been less heavily pretreated.

29/3,AB/27 (Item 6 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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11899299 BIOSIS Number: 98499299

Radiometal labeling of recombinant proteins by a genetically engineered minimal chelatin site: Technetium-99m coordination by single-chain Fv ****antibody**** fusion proteins through a C-terminal cysteinyl peptide

George A J T; Jamar F; Tai M-S; Heelan B T; Adams G P; McCartney J E; Houston L L; Weiner L M; Oppermann H; Peters A M; Huston J S

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Proceedings of the National Academy of Sciences of the United States of America 92 (18). 1995. 8358-8362.

Full Journal Title: Proceedings of the National Academy of Sciences of the United States of America

ISSN: 0027-8424

Language: ENGLISH

Print Number: Biological Abstracts Vol. 100 Iss. 010 Ref. 150156

We describe a method to facilitate radioimaging with technetium-99m (99mTc) by genetic incorporation of a 99mTc chelation site in recombinant single-chain Fv (sFv) **antibody** proteins. This method relies on fusion of the sFv C terminus with a Gly-4Cys peptide that specifically coordinates 99mTc. By using analogues of the 26-10 anti-digoxin sFv as our primary model, we find that addition of the chelate peptide, to form 26-10-1 sFv', does not alter the antigen-binding affinity of sFv. We have demonstrated nearly quantitative chelation of 0.5-50 mCi of 99mTc per mg of 26-10-1 sFv' (1 Ci = 37 GBq). These 99mTc-labeled sFv' complexes are highly stable to challenge with saline buffers, plasma, or diethylenetriaminepentaacetic acid. We find that the 99mTc-labeled **741F8** sFv', specific for the **c**.*erbB** -2 tumor-associated antigen, is effective in imaging human ovarian carcinoma in a scid mouse tumor xenograft model. This fusion chelate methodology should be applicable to diagnostic imaging with 99mTc and radioimmunotherapy with 186Re or 188Re, and its use could extend beyond the sFv' to other engineered **antibodies**, recombinant proteins, and synthetic peptides.

29/3,AB/28 (Item 7 from file: 55)
 DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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11779213 BIOSIS Number: 98379213

Engineering disulfide-linked single-chain Fv dimers ((sFv)-2) with improved solution and targeting properties: Anti-digoxin 26-10 (sFv)-2 and anti-**c**.*erbB** -2 **741F8**** (sFv)-2 made by protein folding and bonded through C-terminal cysteinyl peptides

McCartney J E; Tai M-S; Hudziak R M; Adams G P; Weiner L M; Jin D; Stafford W F III; Liu S; Bookman M A; Laminet A A; Fand I; Houston L L; Oppermann H; Huston J S

Creative BioMolecular Inc., 45 South St., Hopkinton, MA 01748, USA
 Protein Engineering 8 (3). 1995. 301-314.

Full Journal Title: Protein Engineering

ISSN: 0269-2139

Language: ENGLISH

Print Number: Biological Abstracts Vol. 100 Iss. 005 Ref. 071051

Single-chain Fv fusions with C-terminal cysteinyl peptides (sFv) have been engineered using model sFv proteins based upon the 26-10 anti-digoxin IgG and ****741F8**** anti-**c**.*erbB** -2** IgG monoclonal ****antibodies****. As part of the 741F8 sFv construction process, the PCR-amplified 741F8 V-H gene was modified in an effort to correct possible primer-induced errors. Genetic replacement of the N-terminal beta-strand sequence of ****741F8**** V-H with that from the FRI of anti-**c**.*erbB** -2 **520C9**** V-H resulted in a dramatic improvement of sFv folding yields. Folding in urea-glutathione redox buffers produced active sFv' with a protected C-terminal sulphhydryl, presumably as the mixed disulfide with glutathione. Disulfide-bonded (sFv)-2 homodimers were made by disulfide interchange or oxidation after reductive elimination of the blocking group. Both 26-10 (sFv)-2 and ****741F8**** (sFv)-2 existed as stable dimers that were well behaved in solution, whereas ****741F8**** sFv and sFv' exhibited considerable self-association. The ****741F8**** sFv binds to the extracellular domain (ECD) of the **c**.*erbB** -2** oncogene protein, which is often overexpressed in breast cancer and other adenocarcinomas. The recombinant ECD was prepared to facilitate the analysis of ****741F8**** binding site properties; the cloned ECD gene, modified to encode a C-terminal Ser-Gly-His-6 peptide, was transfected into Chinese hamster ovary cells using a vector that also expressed dihydrofolate reductase to facilitate methotrexate amplification. Optimized cell lines expressed ECD-His-6 at high levels in a cell bioreactor; after isolation by immobilized metal affinity chromatography, final ECD yields were as high as 47 mg/l. An animal tumor model complemented physicochemical studies of ****741F8**** species and indicated increased tumor localization of the targeted ****741F8**** (sFv)-2 over other monovalent ****741F8**** species.

29/3,AB/29 (Item 8 from file: 55)
 DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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11554068 BIOSIS Number: 98154068

Physical characterization of recombinant anti-**c**.*erbB** -2 **741F8****

single chain Fv **antibody** dimer (sFv)-2 and analysis of its interaction with recombinant extracellular domain of **c*-erbB*-2** oncogene product by analytical ultracentrifugation

Liu S; Tai M-S; McCartney J; Oppermann H; Hudziak R M; Houston L L; Weiner L M; Huston J S; Stafford W F

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Biophysical Journal 68 (2 PART 2). 1995. A407.

Full Journal Title: 39th Annual Meeting of the Biophysical Society, San Francisco, California, USA, February 12-16, 1995. Biophysical Journal

ISSN: 0006-3495

Language: ENGLISH

Print Number: Biological Abstracts/RRM Vol. 047 Iss. 004 Ref. 056789

29/3,AB/30 (Item 9 from file: 55)

DIALOG(R)File 55:BIOSIS PREVIEWS(R)

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11252100 BIOSIS Number: 97452100

Antigen forks: Bispecific reagents that inhibit cell growth by binding selected pairs of tumor antigens

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Cancer Immunology Immunotherapy 39 (1). 1994. 41-48.

Full Journal Title: Cancer Immunology Immunotherapy

ISSN: 0340-7004

Language: ENGLISH

Print Number: Biological Abstracts Vol. 098 Iss. 008 Ref. 106412

Bispecific **antibodies** of a new category, termed "antigen forks", were constructed by crosslinking **antibodies** that recognized pairs of distinct tumor cell surface antigens. At concentrations of 1-100 nM, several such forks inhibited the growth of human tumor cell lines bearing both relevant antigens. The same cells were not inhibited by unconjugated component **antibodies**, and the active conjugates did not inhibit the growth of human cell lines that expressed lower levels of relevant antigens. The three most active antigen forks all contained monoclonal **antibody** 454A12, which recognizes human transferrin receptor. This **antibody** was conjugated respectively to **antibodies** **113F1** (against a tumor-associated glycoprotein complex), 317G5 (against a 42-kDa tumor-associated glycoprotein), or **520C9** (against the **c*-erbB*-2** protooncogene product). The 317G5-454A12 fork strongly inhibited the HT-29 and SW948 human colorectal cancer cell lines, while the **113F1**-454A12 and **520C9**-454A12 forks strongly inhibited the SK-BR-3 human breast cancer cell line and the **113F1**-454A12 fork was also effective against SW948. By designing forks against antigens of incompatible function that are co-expressed at high levels on tumor cells but not on normal tissues, it may be possible to generate reagents that inhibit tumor growth with enhanced selectivity.

29/3,AB/31 (Item 10 from file: 55)

DIALOG(R)File 55:BIOSIS PREVIEWS(R)

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11220912 BIOSIS Number: 97420912

Gamma-Interferon inhibits Fc receptor II-mediated phagocytosis of tumor cells by human macrophages

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Cancer Research 54 (9). 1994. 2456-2461.

Full Journal Title: Cancer Research

ISSN: 0008-5472

Language: ENGLISH

Print Number: Biological Abstracts Vol. 098 Iss. 007 Ref. 091724

In vitro, monocyte-derived macrophages (MDM) are capable of efficient **antibody**-mediated phagocytosis of human nucleated tumor cells. These MDM express on their cell surface all three classes of Fc receptors for IgG (Fc-gamma-R). Fc-gamma-R specificity for murine **antibody** isotype allowed us to examine the phagocytic role of Fc-gamma-RII on control and gamma-interferon (EFN-gamma)-primed MDM. Monoclonal **antibody** **520C9** (IgG1) mediates phagocytosis through Fc-gamma-RII. This monoclonal

****antibody**** is directed against the ****HER**-*2**** /neu protooncogene product overexpressed on a variety of adenocarcinomas including the breast carcinoma cell line SK-BR-3. Our results showed that IFN-gamma treatment of differentiated MDM (days 8-12 in culture) inhibited Fc-gamma-RII-mediated phagocytosis in a dose-dependent manner with negative effects noted at doses as low as 0.1 units/ml. The percentage reduction in ****antibody****-mediated phagocytosis observed following IFN-gamma priming (40 units/ml for 18 h) ranged from 23-89% of control. The inhibitory effect was evident when exposure to IFN-gamma was transient. Fc-gamma-RII expression was not altered by IFN-gamma treatment. In our model, IFN-gamma did not up-regulate or down-regulate ****HER**-*2**** /neu protein expression on our targets or affect the level of CD14 antigen expression on our MDM. Although IFN-gamma is a potent activator of monocytes/macrophages and can enhance certain tumoricidal mechanisms, our data show that ****antibody****-dependent phagocytosis through the type II Fc receptor is inhibited by IFN-gamma priming. Nonspecific phagocytosis was not affected.

29/3,AB/32 (Item 11 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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10925896 BIOSIS Number: 97125896

Immunohistochemical detection of ****c**-*erbB** -2** expression by neoplastic human tissue using monospecific and bispecific monoclonal ****antibodies****

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Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA 19111, USA
International Journal of Biological Markers 8 (4). 1993. 233-239.
Full Journal Title: International Journal of Biological Markers
ISSN: 0393-6155
Language: ENGLISH

Print Number: Biological Abstracts Vol. 097 Iss. 006 Ref. 075756

Selected murine monoclonal ****antibodies**** (M Ab) have been shown to inhibit relevant tumor growth in vitro and in animal models. Recently, bispecific ****antibodies**** (BsMAB) have been developed which target cytolytic effector cells via one ****antibody**** binding site and tumor antigen by the other specificity. For example, the BsM Ab ****2B1**** possesses specificity for ****c**-*erbB** -2** and Fc-gamma-RIII, the low affinity Fc-gamma receptor expressed by polymorphonuclear leukocytes (PMN), macrophages and large granular lymphocytes (LGL). The human homologue of the rat neu oncogene, ****c**-*erbB** -2**, has been demonstrated to be amplified in breast, gastrointestinal, lung and ovarian carcinomas. Tumor expression of ****c**-*erbB** -2** has been shown to be an important prognostic indicator in breast and ovarian carcinomas. The restricted expression of the ****c**-*erbB** -2** protooncogene product in normal human tissues and the wide distribution of ****c**-*erbB** -2** expression in such tumors may justify attempts to use an appropriately constructed Bs M Ab in clinical trials. In this report we have addressed this issue by immunohistochemically evaluating the expression of ****c**-*erbB** -2** oncogene product in a variety of malignant tumors utilizing ****2B1**** and the anti-****c**-*erbB** -2** monovalent parent of ****2B1****, ****520C9****. Among the studied neoplasms, ****c**-*erbB** -2** expression was detected in 49% of primary carcinomas stained with ****520C9**** and in 39% of those stained with ****2B1****. In the group of metastatic tumors, ****c**-*erbB** -2** oncoprotein was detected in 52% of cases by ****520C9**** and in 41% by ****2B1****. Our results indicate that immunocytochemistry using bispecific monoclonal ****2B1**** is a reliable method for the detection of ****c**-*erbB** -2** expression, and that this Bs M Ab detects ****c**-*erbB** -2** expression in tumors nearly as well as its anti-****c**-*erbB** -2** monovalent parent ****antibody****.

29/3,AB/33 (Item 12 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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10514326 BIOSIS Number: 96114326

BINDING AND CYTOTOXICITY CHARACTERISTICS OF THE BISPECIFIC MURINE MONOCLONAL ****ANTIBODY** *2B1****
WEINER L M; HOLMES M; RICHESON A; GODWIN A; ADAMS G P; HSIEH-MA S T; RING D B; ALPAUGH R K
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J IMMUNOL 151 (5). 1993. 2877-2886. CODEN: JOIMA

Full Journal Title: Journal of Immunology

Language: ENGLISH

Bispecific monoclonal **antibodies** (BsmAb) with specificity for tumor Ag and effector cell trigger molecules have been shown to redirect the cytotoxicity of several peripheral blood mononuclear cell populations against relevant tumor. The BsmAb, **2B1**, binds to the extracellular domain of the **c-erbB-2** gene product of the **HER2** /neu protooncogene and to CD16. In this report, the binding and cytotoxic characteristics of **2B1** are presented. Maximal saturation binding of **2B1** to PBL and **c-erbB-2** expressing SK-OV-3 cells occurred in the 1 .mu.g/ml concentration range. However, substantial lysis potentiation was observed at 1000-fold lower BsmAb concentrations. Optimal tumor lysis was obtained when the BsmAb, PBL, and target cells were continuously coincubated. When PBL were franked with **2B1**, washed, and added to labeled targets, substantially less lysis was observed. These results suggest that the best way to therapeutically exploit the cytotoxic attributes of **2B1** may be to obtain continuous BsmAb exposure to tumor. Approaches based on franking of this BsmAb to PBL may not be warranted.

29/3,AB/34 (Item 13 from file: 55)

DIALOG(R)File 55:BIOSIS PREVIEWS(R)

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10514086 BIOSIS Number: 96114086

HIGHLY SPECIFIC IN-VIVO TUMOR TARGETING BY MONOVALENT AND DIVALENT FORMS OF **741F8** ANTI-**c-erbB-2** SINGLE CHAIN FV

ADAMS G P; MCCARTNEY J E; TAI M-S; OPPERMAN H; HUSTON J S; STAFFORD W F III; BOOKMAN M A; FAND I; HOUSTON L L; WEINER L M

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CANCER RES 53 (17). 1993. 4026-4034. CODEN: CNREA

Full Journal Title: Cancer Research

Language: ENGLISH

The in vivo properties of monovalent and divalent single-chain Fv (sFv)-based molecules with the specificity of the anti-**c-erbB-2** monoclonal **antibody** **741F8** were examined in scid mice bearing SK-OV-3 tumor xenografts. **741F8** sFv monomers exhibited rapid, biphasic clearance from blood, while a slightly slower clearance was observed with the divalent **741F8** (sFv)₂ comprising a pair of **741F8** sFv with a C-terminal Gly4Cys joined by a disulfide bond. Following i.v. injection, the **741F8** sFv monomer was selectively retained in **c-erbB-2**-overexpressing SK-OV-3 tumor, with excellent tumor:normal organ ratios uniformly exceeding 10:1 by 24 h. The specificity of this effect was demonstrated by the lack of retention of the anti-digoxin 26-10 sFv monomer, as evaluated by biodistribution studies, gamma camera imaging, and cryomacroautoradiography studies. The specificity index (**741F8** sFv retention/26-10 sFv retention) of **741F8** monomer binding, measured by the percentage of injected dose per g of tissue, was 13.2:1 for tumor, and 0.8 to 2.1 for all tested normal organs, with specificity indices for tumor:organ ratios ranging from 7.0 (kidneys) to 16.7 (intestines). Comparing divalent **741F8** (sFv)₂ with the 26-10 (sFv)₂, similar patterns emerged, with specificity indices for retention in tumor of 16.9 for the Gly4Cys-linked (sFv)₂. These data demonstrate that, following their i.v. administration, both monovalent and divalent forms of **741F8** sFv are specifically retained by SK-OV-3 tumors. This antigen-specific binding, in conjunction with the 26-10 sFv controls, precludes the possibility that passive diffusion and pooling in the tumor interstitium contributes significantly to long-term tumor localization. **741F8** (sFv)₂ species with peptide spacers exhibited divalent binding and increased retention in tumors as compared with **741F8** sFv monomers. Since the blood retention of the (sFv)₂ is slightly more prolonged than that of the monomer, it was necessary to demonstrate that the increased tumor localization of the peptide-linked (sFv)₂ was due to its divalent nature. The significantly greater localization of the divalent bismalimidohexane-linked **741F8** (sFv)₂ as compared with a monovalent **741F8** Fab fragment of approximately the same size suggests that the increased avidity of the (sFv)₂ is a factor in its improved tumor retention. This is the first report of successful specific in vivo targeting of tumors by divalent forms of sFv molecules. The improved retention of specific divalent (sFv)₂ by tumors may have important

consequences for targeted diagnostic or therapeutic strategies.

29/3,AB/35 (Item 14 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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10259549 BIOSIS Number: 45059549
BINDING CHARACTERISTICS OF THE BISPECIFIC MONOCLONAL **ANTIBODY** **2B1**
GARCIA DE PALAZZO I E; RICHESON A; WEINER L M
FOX CHASE CANCER CENT., PHILADELPHIA, PA 19111, USA.
84TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH,
ORLANDO, FLORIDA, USA, MAY 19-22, 1993. PROC AM ASSOC CANCER RES ANNU MEET
34 (0). 1993. 477. CODEN: PAMRE
Language: ENGLISH
Document Type: CONFERENCE PAPER

29/3,AB/36 (Item 15 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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10052477 BIOSIS Number: 95052477
A HUMAN TUMOR XENOGRAFT MODEL OF THERAPY WITH A BISPECIFIC MONOCLONAL
ANTIBODY TARGETING **c**-**erbB**-2 AND CD16
WEINER L M; HOLMES M; ADAMS G P; LACRETA F; WATTS P; DE PALAZZO I G
DEP. MED. ONCOL., FOX CHASE CANCER CENTER, 7701 BURHOLME AVE.,
PHILADELPHIA, PA. 19111.
CANCER RES 53 (1). 1993. 94-100. CODEN: CNREA
Full Journal Title: Cancer Research
Language: ENGLISH
New strategies are required to clinically exploit the ability of
monoclonal **antibodies** to target tumor for lysis by cellular effector
mechanisms. In this report we examine the therapeutic effects of **2B1**, a
bispecific monoclonal **antibody** with specificity for the extracellular
domain of the **c**-**erbB**-2 oncogene product and the human Fc.gamma.
receptor, Fc.gamma.RIII (CD16), describe the characteristics and
limitations of this model, and examine the mechanisms underlying the
observed responses. The model uses SK-OV-3 human ovarian carcinoma
xenografts in scid mice. These cells are susceptible to **2B1**-directed
lysis by human peripheral blood lymphocytes or lymphokine-activated killer
cells, and maintain **c**-**erbB**-2 expression in vivo, 125I-labeled
2B1 selectively accumulates in tumor, with a peak of 10.5% injected
dose/g of tumor 24 h following its i.v. injection. However, the selectivity
of this binding is lessened by **2B1** accumulation in the lungs and other
normal organs and persistence in the blood. This is caused by **antibody**
binding to murine lung, colon, stomach, and skin expressing the epitope
recognized by the anti-**c**-**erbB**-2 component of **2B1** in
tumor-bearing, but not normal mice. In treatment studies using various
permutations of **antibody**, human peripheral blood lymphocytes or
lymphokine-activated killer cells and interleukin 2, cellular therapy alone
had minimal effects on SK-OV-3 xenograft growth, but significantly improved
when **2B1** treatment was incorporated. Median survivals increased from 80
+/- 3.5 days with no therapy to 131 +/- 7.3 days following therapy with
100 .mu.g **2B1**, interleukin 2, and human peripheral blood lymphocytes,
with 70% of animals exhibiting no evidence of tumor at day 150. These
effects were preserved when the cells were administered in human serum. In
contrast, human serum abolished the antitumor effects of **520C9**, which
is the parent anti-**c**-**erbB**-2 **antibody** of **2B1**. Thus **2B1**
-based therapy has therapeutic effects, without obvious toxicity, despite
the targeting of this **antibody** to normal murine tissues. Since
combination of **2B1** and interleukin 2 may have antitumor properties,
mechanisms other than bispecific monoclonal **antibody**-promoted
conjugation of **c**-**erbB**-2 antigen-expressing tumor to CD16-expressing
effector cells may be involved.

29/3,AB/37 (Item 16 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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10052460 BIOSIS Number: 95052460

IN-VITRO CYTOTOXIC TARGETING BY HUMAN MONONUCLEAR CELLS AND BISPECIFIC
ANTIBODY **2B1** RECOGNIZING **C**-*erbB*-2 PROTOONCOGENE PRODUCT AND
FC-GAMMA RECEPTOR III

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CANCER RES 52 (24). 1992. 6832-6839. CODEN: CNREA
Full Journal Title: Cancer Research
Language: ENGLISH

Bispecific murine monoclonal **antibody** **2B1**, possessing dual specificity for the human **C**-*erbB*-2 protooncogene product and human Fc.gamma. receptor III (CD16) was evaluated for the ability to promote specific lysis of **C**-*erbB*-2-positive tumor cells in vitro. In short-term 51Cr release assays with human mononuclear cells as effectors and SK-Br-3 human breast cancer cells as targets, neither parental **antibody** of **2B1** mediated significant specific lysis, but bispecific **antibody** was as active as a chemical heteroconjugate, with 5 ng/ml of **2B1** causing half-maximal lysis at an effector/target ratio of 20:1 and 2 ng/ml **2B1** causing half-maximal lysis at an E/T ratio of 40:1. The cytotoxic targeting activity of **2B1** F(ab')₂ fragment was the same as that of whole bispecific **antibody**, and the activity of whole **2B1** was not reduced when assays were performed in 100% autologous human serum, indicating that **2B1** binds effector cells through the CD16-binding site derived from parental **antibody** 3G8 rather than through its Fc portion. Variable inhibition of **2B1** mediated lysis was observed when autologous polymorphonuclear leukocytes from different donors were added to mononuclear effector cells at a 2:1 ratio; this inhibition was overcome at higher **antibody** concentration. **2B1** bispecific monoclonal **antibody** was also able to mediate targeted cytolysis using whole human blood as a source of effector cells or using effector or target cells derived from ovarian cancer patients.

29/3,AB/38 (Item 17 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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9593438 BIOSIS Number: 94098438
A COMBINATION OF TWO IMMUNOTOXINS EXERTS SYNERGISTIC CYTOTOXIC ACTIVITY
AGAINST HUMAN BREAST-CANCER CELL LINES
CREWS J R; MAIER L A; YU Y H; HESTER S; O'BRIANT K; LESLIE D S; DESOMBRE
K; GEORGE S L; BOYER C M; ET AL
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27710.

INT J CANCER 51 (5). 1992. 772-779. CODEN: IJCNA
Full Journal Title: International Journal of Cancer
Language: ENGLISH

In previous studies, combinations of immunotoxins reactive with different cell-surface antigens have exerted additive cytotoxicity against tumor cells in culture. In this report we describe a combination of 2 immunotoxins that produce synergistic cytotoxic activity. Recombinantly derived ricin A chain (RTA) was conjugated with murine monoclonal **antibodies** (MAbs) 317G5, 260F9, 454A12 and **741F8** that bound to cell-surface determinants of 42, 55, 180 (transferrin receptor) and 185 kDa (**HER**-*2* / neu) expressed by the SKBr3 human breast-cancer cell line. When inhibition of clonogenic growth was measured in a limiting dilution assay, the combination of 260F9-RTA and 454A12-RTA produced synergistic cytotoxic activity against SKBr3 and 2 other breast-cancer cell lines. All other combinations produced only additive inhibition of clonogenic growth. Simultaneous binding of 260F9 and 454A12 was not supra-additive, but sub-populations of cells which lacked one or the other antigen could be detected. Kinetic studies of internalization, using **antibodies** conjugated with gold particles, indicated that 454A12 remained within peripheral endosomes for a longer interval in the presence of 260F9. This change in the traffic of the transferrin receptor may contribute to synergy between 260F9-RTA and 454A12-RTA.

29/3,AB/39 (Item 18 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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8647589 BIOSIS Number: 92112589

SELECTION OF HYBRID HYBRIDOMAS BY FLOW CYTOMETRY USING A NEW COMBINATION OF FLUORESCENT VITAL STAINS

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J IMMUNOL METHODS 141 (2). 1991. 165-176. CODEN: JIMMB

Full Journal Title: Journal of Immunological Methods

Language: ENGLISH

A new combination of fluorescent dye (rhodamine 123 and hydroethidine) was used to internally label hybridoma fusion partners. Murine hybridoma **520C9** (recognizing human **c*-**erbB** -2) was labeled with hydroethidine. Murine hybridoma 3G8 (recognizing human Fc.gamma. receptor III) was labeled with rhodamine 123, and verapamil was used to block rhodamine efflux of via P-glycoprotein. Viability assays showed little cytotoxicity from these dyes at the concentrations used. The labeled cells were fused with polyethylene glycol, sorted for dual fluorescence on an Epics V cell sorter, and cloned. Hybrid hybridomas producing bispecific **antibodies** were selected for ability to promote lysis of SK-Br-3 breast cancer cells by human mononuclear cells. Several positive clones were obtained and shown to have a double content of DNA. Bispecific **antibody** produced by subclone **2B1** was purified by anion exchange chromatography and shown to bind both tumor cells and Fc.gamma.R III bearing cells. Using two parameter flow cytometric analysis, we were able to measure a 'bridging' effect of this bispecific **antibody**, which caused formation of complexes between PMNs and SK-Br-3 cells. Either parental **antibody** could compete with bispecific **antibody** to block such complexing. This function method provides several advantages over other techniques presently used (speed, convenience, low toxicity and automatic exclusion of dead cells) and can be applied to produce other hybrid hybridomas.

29/3,AB/40 (Item 19 from file: 55)
DIALOG(R)File 55:BIOSIS PREVIEWS(R)
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8636879 BIOSIS Number: 92101879

IDENTITY OF BCA200 AND **C*-**ERBB** -2 INDICATED BY REACTIVITY OF MONOCLONAL **ANTIBODIES** WITH RECOMBINANT **C*-**ERBB** -2

RING D B; CLARK R; SAXENA A
DEP. IMMUNOL., CETUS CORPORATION, EMERYVILLE, CALIF. 94068.
MOL IMMUNOL 28 (8). 1991. 915-918. CODEN: MOIMD

Full Journal Title: Molecular Immunology

Language: ENGLISH

BCA200 has been described as a 200,000 Mr monomeric cell surface glycoprotein associated with human breast cancer. Since the physical properties and cellular distribution of BCA200 resemble those of **c*-**erbB** -2, **antibodies** to BCA200 were tested for the ability to bind a recombinant protein containing the **c*-**erbB** -2 extracellular domain (erbB-2 ECD). Three **antibodies** to distinct epitopes of BCA200 reacted with erbB-2 ECD but not with a control protein expressed in a similar baculovirus lysate. Control myeloma proteins and **antibodies** to four other antigens did not react with erbB-2 ECD. A protein with the expected molecular weight for erbB-2 ECD was also immunoprecipitated by anti-BCA200 **antibody** **520C9**. We conclude that BCA200 is another synonym for **c*-**erbB** -2.

29/3,AB/41 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
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10042836 EMBASE No: 96225074

In vitro tumor growth inhibition by bispecific **antibodies** to human transferrin receptor and tumor-associated antigens is augmented by the iron chelator deferrioxamine

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Clinical Immunology and Immunopathology (USA) , 1996, 80/2 (185-193)

CODEN: CLIIA ISSN: 0090-1229

LANGUAGES: English SUMMARY LANGUAGES: English

Previously, a panel of mouse monoclonal **antibodies** (mAbs) to several

tumor-associated antigens was chemically crosslinked to an IgG1 anti-human transferrin receptor **antibody**, 454A12. We called this new class of bispecific **antibodies** (BmAbs) 'antigen forks' and showed that these antigen forks inhibited but did not completely prevent tumor cell growth. We speculated that the conjugates acted by heterologously crosslinking two antigens in a manner that interfered with the functions of one or both. The most effective BmAbs all shared one specificity for the human transferrin receptor. A monoclonal **antibody** to this receptor has been shown by others to reduce tumor cell growth when used with the iron chelator deferoxamine. When we combined our antigen forks with deferoxamine, two of five BmAbs synergized with deferoxamine to arrest tumor; cell count at or below input levels. The most effective BmAbs were 317G5/454A12 (3/4) and **520C9** /454A12 (5/4). mAb 317G5 recognizes a 42-kDa tumor associated glycoprotein, and mAb **520C9** recognizes the **c-erbB**-2 protooncogene product. BmAb 3/4 was most effective against colorectal cancer cell line HT-29, and BmAb 5/4 was most effective against breast cancer cell line SEC-BR-3. When deferoxamine and BmAb were replaced by fresh medium after a 6- or 7-day treatment period, no regrowth of tumor cells was observed during the next 4 days, although regrowth was seen if either deferoxamine or BmAb was used alone. Our results show that BmAbs with specificities for transferrin receptor and certain tumor-associated antigens effectively inhibit tumor growth in vitro. When used in combination with deferoxamine, such BmAbs may have therapeutic potential for the treatment of cancer.

29/3,AB/42 (Item 2 from file: 73)
 DIALOG(R)File 73:EMBASE
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9992086 EMBASE No: 96157946
 Targeting growth factor receptors with bispecific molecules
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 Current Medicinal Chemistry (Netherlands) , 1996, 3/2 (87-100) CODEN: CMCHE ISSN: 0929-8673
 LANGUAGES: English SUMMARY LANGUAGES: English
 Peptide growth factor receptors on the surface of malignant cells bind to their ligands with high affinity, resulting in intracellular responses which cause differentiation, growth, and the survival of these cells. Peptide growth factors, or monoclonal **antibodies** (mAbs) which target growth factor receptors, have been conjugated to drugs, toxins, radionuclides, or other mAbs that recognize/activate effector cells which can phagocytose or kill. These types of conjugated products, which have the ability to kill malignant cells, we call bispecific molecules (BsMol) and is the basis of this review article. The growth factors/receptors covered include alpha - and beta-melanocyte stimulating hormone (MSH), bombesin/gastrin releasing peptide (BN/GRP), epidermal growth factor (EGF), **HER**-**2**/neu oncogene protein (**p185**(**HER2**)), interleukin-2, and somatostatin. The preparation and biological use/activity of the following BsMol are discussed: beta-MSH-daunomycin, (Nle4, D-Phe7)MSH-anti-CD3, 111In-DTPA-bis-alpha-MSH, DAB389-MSH, mAb22-Lys-BN, mAb22-Antag1, anti-EGFR/anti-CD3, DOXER2, DAB389EGF, 111In-DTPA-225, anti-**p185**(**HER2**)-SAP, **2B1**, MDX-210, humAb4D5-8 x humAbUCHT1, DAB486IL-2, 111In-DTPA-octreotide (OctreoScan (R)), OX-26-NGF, and IVA039.1.

29/3,AB/43 (Item 3 from file: 73)
 DIALOG(R)File 73:EMBASE
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9970797 EMBASE No: 96146994
 Human neutrophil interactions of a bispecific monoclonal **antibody** targeting tumor and human FcgammaRIII
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 Cancer Immunology Immunotherapy (Germany) , 1996, 42/3 (141-150) CODEN: CIIMD ISSN: 0340-7004
 LANGUAGES: English SUMMARY LANGUAGES: English

****2B1**** is a bispecific murine monoclonal ****antibody**** (bsmAb) targeting the ****c**-*erbB****-2 and CD16 (FcgammaRIII) antigens. ****c**-*erbB****-2 is over-expressed by a variety of adenocarcinomas, and CD16, the low-affinity Fcgamma receptor for aggregated immunoglobulins, is expressed by polymorphonuclear leukocytes (PMN), natural killer (NK) cells and differentiated mononuclear phagocytes. ****2B1**** potentiates the in vitro lysis of ****c**-*erbB****-2 over-expressing tumors by NK cells and macrophages. In this report, the interactions between ****2B1**** and PMN were investigated to assess the impact of these associations on in vitro ****2B1****-promoted tumor cytotoxicity by human NK cells. The peak binding of ****2B1**** to PMN was observed at a concentration of 10 microg/ml ****2B1****. However, ****2B1**** rapidly dissociated from PMN in vitro at 37degreeC in non-equilibrium conditions. This dissociation was not caused by CD16 shedding. When PMN were labeled with 125I-****2B1**** and incubated at 37degreeC and the supernatants examined by HPLC analysis, the Fab regions of dissociated ****2B1**** were not complexed with shed CD16 extracellular domain. While most of the binding of ****2B1**** to PMN was solely attributable to Fab-directed binding to FcgammaRIII, PMN-associated ****2B1**** also bound through Fcgamma-domain/FcgammaRII interactions. ****2B1**** did not promote in vitro PMN cytotoxicity against ****c**-*erbB****-2-expressing SK-OV-3 tumor cells. When PMN were coincubated with peripheral blood lymphocytes, SK-OV-3 tumor and ****2B1****, the concentration of ****2B1**** required for maximal tumor lysis was lowered. Although PMN may serve as a significant competitive binding pool of systemically administered ****2B1**** in vivo, the therapeutic potential of the targeted cytotoxicity properties of this bsmAb should not be compromised.

29/3,AB/44 (Item 4 from file: 73)
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9819462 EMBASE No: 95372895

Enhanced tumor specificity of ****741F8****-1 (sFv)2, an anti-****c**-*erbB****-2 single-chain Fv dimer, mediated by stable radioiodine conjugation
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Journal of Nuclear Medicine (USA), 1995, 36/12 (2276-2281) CODEN: JNMEA
 ISSN: 0161-5505

LANGUAGES: English SUMMARY LANGUAGES: English

The goal of this study was to determine if the stabilization of the radioiodine-protein bond by the N-succinimidyl p-iodobenzoate (PIB) method improved the degree and specificity of tumor localization of 125I-****741F8****-1 (sFv)2, an anti-****c**-*erbB****-2 sFv dimer, in an immunodeficient murine model. Methods: Gamma camera images were acquired 21 hr after intravenous administration of 131I-****741F8****-1 (sFv)2 labeled by the p-iodobenzoate or chloramine T methods. The stability of the radioiodine-protein bond also was assessed in plasma samples after intravenous injection of 125I-****741F8****-1 (sFv)2 labeled by either the chloramine T or p-iodobenzoate methods. Results: By 6 hr postinjection, 97% of the activity associated with the 125I-****741F8****-1 (sFv)2 labeled by the p-iodobenzoate method was protein bound compared with 61% after labeling with the chloramine-T method. These observations indicate that increasing the stability of the conjugation between the radioiodine and the sFv molecule can significantly increase the degree and specificity of tumor targeting. Significantly greater tumor retention (p < 0.005) and lower blood (p < 0.001), spleen (p < 0.001) and stomach (p < 0.005) retention were observed in biodistribution studies when the p-iodobenzoate conjugate was used. This resulted in superior tumor-to-organ ratios for all tissue samples studied. Conclusion: These observations may have clinical relevance for the use of radiolabeled sFv as imaging agents.

29/3,AB/45 (Item 5 from file: 73)
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9802769 EMBASE No: 95346479

Preclinical studies and optimization of specific tumor delivery for ****741F8**** sFv, an anti-****c**-*erbB****-2 single-chain species

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International Journal of Pharmacognosy (Netherlands) , 1995, 33/SUPPL. (75-91) CODEN: IJPYE ISSN: 0925-1618

LANGUAGES: English SUMMARY LANGUAGES: English

Single-chain Fv fragments (sFv) that bind tumor-associated antigens exhibit highly specific tumor targeting characteristics based on studies of intravenous sFv administration to immunodeficient mice bearing human tumor xenografts. These features suggest that sFv may be used as targeting vehicles for diverse agents such as radionuclides, toxins, chemotherapeutic agents or genes. However, the quantitative tumor retention of sFv molecules is lowered by their fast clearance and rapid dissociation from antigen. We have found that the retention of tumor-specific **741F8** sFv in tumor is an antigen-specific event and not the result of extravascular pooling. We also have produced divalent **741F8** (sFv)₂ species and have examined their pharmacokinetics and biodistribution properties in relevant tumor-bearing mice. Finally we have employed a number of strategies to optimize the tumor-specific retention of radiolabeled sFv molecules; escalations in administered doses, repetitive intravenous bolus administrations, subcutaneous continuous infusions and the use of an alternate labeling system.

29/3,AB/46 (Item 6 from file: 73)

DIALOG(R)File 73:EMBASE

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9792163 EMBASE No: 95353423

Targeting **c**.*erbB** -2 expressing tumors using single-chain Fv monomers and dimers

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Cancer Research (USA) , 1995, 55/23 SUPPL. (5983s-5989s) CODEN: CNREA
ISSN: 0008-5472

LANGUAGES: English SUMMARY LANGUAGES: English

Single-chain Fv proteins containing a COOH-terminal cysteine (sFv') were constructed by using an antidigoxin 26-10 sFv and an anti-**c**.*erbB** -2 **741F8** sFv. The fully active sFv' proteins were prepared by expression in Escherichia coli as insoluble inclusion bodies, followed by in vitro refolding using glutathione redox buffers and purification. The COOH-terminal cysteines of the refolded sFv' proteins were protected by a blocking group presumed to be the glutathionyl peptide, which was easily and selectively removed by gentle reduction. Air oxidation of the reduced sFv' monomers resulted in the efficient formation of disulfide-linked sFv' homodimers, designated (sFv')₂, which were stable under oxidizing conditions and relatively slow to be disrupted under reducing conditions. The (26-10-1 sFv)-(**741F8** -1 sFv) heterodimer was prepared and possessed dual-antigen specificity: the active bispecific (sFv')₂ dimerized under native conditions, apparently as a manifestation of self-association by the **741F8** sFv' subunit. Biodistribution and imaging studies that were performed on mice bearing human SK-OV-3 tumor xenografts that express the **c**.*erbB** -2 as a cell surface antigen were reviewed. Radioiodinated **741F8** -2 (sFv')₂ homodimer localized to the tumors with high specificity, as evidenced by excellent tumor:normal tissue ratios. Sagittal section autoradiography of whole animals 24 h after administration of **antibody** species revealed that **741F8** (sFv')₂ produced a stronger tumor image than comparable doses of the **741F8** Fab, monomeric sFv', and the 26-10 (sFv')₂ control without the high nonspecific background distribution of the **741F8** IgG.

29/3,AB/47 (Item 7 from file: 73)

DIALOG(R)File 73:EMBASE

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9776471 EMBASE No: 95336582

Clinical development of **2B1**, a bispecific murine monoclonal

****antibody**** targeting ****C****-****erbB****-2 and FcgammaRIII

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Journal of Hematotherapy (USA) , 1995, 4/5 (453-456) CODEN: JOEME
ISSN: 1061-6128

LANGUAGES: English SUMMARY LANGUAGES: English

Bispecific monoclonal ****antibodies**** (BsmAb) can be used to specifically target tumor cells for cytotoxicity mediated by defined effector cells. One such BsmAb, ****2B1****, targets the extracellular domains of both the ****c****-****erbB****-2 protein product of the ****HER****-****2**** /neu oncogene and FcgammaRIII (CD16), the Fcgamma receptor expressed by human natural killer cells, neutrophils, and differentiated mononuclear phagocytes. ****2B1**** promotes the conjugation of cells expressing these target antigens. It efficiently promotes the specific lysis of tumor cells expressing ****c****-****erbB****-2 by human NK cells and macrophages over a broad concentration range. ****2B1**** selectively targets ****c****-****erbB****-2-positive human tumor xenografts growing in immunodeficient SCID mice. Treatment of such mice with ****2B1**** plus interleukin 2 (IL-2) inhibits the growth of early, established human tumor xenografts overexpressing ****c****-****erbB****-2. A phase I clinical trial of ****2B1**** has been initiated to determine the toxicity profile and maximum tolerated dose (MTD) of this BsmAb and to examine the biodistribution of the ****antibody**** and the biologic effects of treatment. Preliminary results of this trial indicate that the dose-limiting toxicity for patients with extensive prior bone marrow-toxic therapy is thrombocytopenia for as yet undetermined reasons. Toxicities of fevers, rigors, and associated constitutional symptoms are explained, in part, by treatment-induced systemic expression of cytokines, such as tumor necrosis factor-alpha. Circulating, functional BsmAb is easily detectable in treatment patients' sera and exhibits complex elimination patterns. HAMA and anti-idiotypic treatment-induced ****antibodies**** are induced by ****2B1**** treatment. Some preliminary indications of clinical activity have been observed. BsmAb therapy targeting tumor antigens and FcgammaRIII has potent immunologic effects. Future studies will include the development of more relevant animal models for BsmAb therapy targeting human FcgammaRIII. The ongoing phase I trial will be completed to identify the MTD for patients without extensive prior bone marrow-toxic chemotherapy and radiation. A phase II clinical trial of ****2B1**** therapy in women with metastatic breast cancer is planned, as is a phase I trial incorporating treatment with both ****2B1**** and IL-2.

29/3,AB/48 (Item 8 from file: 73)
DIALOG(R)File 73:EMBASE
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9776465 EMBASE No: 95336576

G-CSF-stimulated PMN in immunotherapy of breast cancer with a bispecific ****antibody**** to FcgammaRI and to ****HER****-****2****/neu (MDX-210)

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Journal of Hematotherapy (USA) , 1995, 4/5 (415-421) CODEN: JOEME
ISSN: 1061-6128

LANGUAGES: English SUMMARY LANGUAGES: English

Myeloid cells can mediate tumor cell cytotoxicity via certain receptors for immunoglobulins. Among the different Fc receptors, the high-affinity IgG receptor (FcgammaRI, CD64) is a promising trigger molecule because it is selectively expressed on effector cells, including monocytes/macrophages and granulocyte colony-stimulating factor (G-CSF)-primed neutrophils. In vitro, a bispecific ****antibody**** (BsAb) (MDX-210, constructed by chemically cross-linking F(ab') fragments of monoclonal ****antibody**** (mAb) ****520C9**** to ****HER****-****2****/neu and F(ab') fragments of mAb 22 to FcgammaRI) mediated effective lysis of ****HER****-****2****/neu overexpressing breast cancer cell lines. ****HER****-****2****/neu (****c****-****erbB2****) is overexpressed in approximately 30% of breast and ovarian carcinomas and is a target for immunotherapy in clinical trials. In vitro assays showed FcgammaRI-positive neutrophils to constitute a major effector cell population during G-CSF therapy. Based on these preclinical data and a preceding study at Dartmouth (New Hampshire) with a single dose of MDX-210 alone, a combination of G-CSF and MDX-210 is tested in a phase I study in breast cancer patients. In this

study, patients receiving G-CSF are treated with escalating single doses of MDX-210. This therapy was generally well tolerated by the treated patients, some of whom reacted with fever and short periods of chills, which were temporally related to elevated plasma levels of IL-6 and TNF-alpha. After MDX-210 application, a transient decrease in the total white blood count and absolute neutrophil count (ANC) was observed. During G-CSF application, isolated neutrophils were highly cytotoxic in the presence of MDX-210 in vitro. These data indicate a potential role for G-CSF and BsAb in immunotherapy.

29/3,AB/49 (Item 9 from file: 73)
DIALOG(R)File 73:EMBASE
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9762008 EMBASE No: 95313186

Phase I trial of **2B1**, a bispecific monoclonal **antibody** targeting **c*-erbB*-2 and FcgammaRIII

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Cancer Research (USA), 1995, 55/20 (4586-4593) CODEN: CNREA ISSN: 0008-5472

LANGUAGES: English SUMMARY LANGUAGES: English

2B1 is a bispecific murine monoclonal **antibody** (BsMAb) with specificity for the **c*-erbB*-2 and FcgammaRIII extracellular domains. This BsMAb promotes the targeted lysis of malignant cells overexpressing the **c*-erbB*-2 gene product of the **HER2**/neu proto-oncogene by human natural killer cells and mononuclear phagocytes expressing the FcgammaRIII A isoform. In a Phase I clinical trial of **2B1**, 15 patients with **c*-erbB*-2-overexpressing tumors were treated with 1 h i.v. infusions of **2B1** on days 1, 4, 5, 6, 7, and 8 of a single course of treatment. Three patients were treated with daily doses of 1.0 mg/m2, while six patients each were treated with 2.5 mg/m2 and 5.0 mg/m2, respectively. The principal non-dose-limiting transient toxicities were fevers, rigors, nausea, vomiting, and leukopenia. Thrombocytopenia was dose limiting at the 5.0 mg/m2 dose level in two patients who had received extensive prior myelosuppressive chemotherapy. Murine **antibody** was detectable in serum following **2B1** administration, and its bispecific binding properties were retained. The pharmacokinetics of this murine **antibody** were variable and best described by nonlinear kinetics with an average t(one-half) of 20 h. Murine **antibody** bound extensively to all neutrophils and to a proportion of monocytes and lymphocytes. The initial **2B1** treatment induced more than 100-fold increases in circulating levels of tumor necrosis factor-alpha, interleukin 6, and interleukin 8 and lesser rises in granulocyte-monocyte colony-stimulating factor and IFN-gamma. Brisk human anti-mouse **antibody** responses were induced in 14 of 15 patients. Several minor clinical responses were observed, with reductions in the thickness of chest wall disease in one patient with disseminated breast cancer. Resolution of pleural effusions and ascites, respectively, were noted in two patients with metastatic colon cancer, and one of two liver metastases resolved in a patient with metastatic colon cancer. Treatment with **2B1** BsMAb has potent immunological consequences. The maximum tolerated dose and Phase II daily dose for patients with extensive prior myelosuppressive chemotherapy was 2.5 mg/m2. Continued dose escalation is required to identify the maximally tolerated dose for patients who have been less heavily pretreated.

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DIALOG(R)File 73:EMBASE
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9714579 EMBASE No: 95268114

Radiometal labeling of recombinant proteins by a genetically engineered minimal chelation site: Technetium-99m coordination by single-chain Fv **antibody** fusion proteins through a C-terminal cysteinyl peptide

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Proceedings of the National Academy of Sciences of the United States of America (USA) , 1995, 92/18 (8358-8362) CODEN: PNAS ISSN: 0027-8424
LANGUAGES: English SUMMARY LANGUAGES: English

We describe a method to facilitate radioimaging with technetium-99m (99mTc) by genetic incorporation of a 99mTc chelation site in recombinant single-chain Fv (sFv) **antibody** proteins. This method relies on fusion of the sFv C terminus with a Gly4Cys peptide that specifically coordinates 99mTc. By using analogues of the 26-10 anti-digoxin sFv as our primary model, we find that addition of the chelate peptide, to form 26-10-1 sFv', does not alter the antigen-binding affinity of sFv. We have demonstrated nearly quantitative chelation of 0.5-50 mCi of 99mTc per mg of 26-10-1 sFv' (1 Ci = 37 GBq). These 99mTc-labeled sFv' complexes are highly stable to challenge with saline buffers, plasma, or diethylenetriaminepentaacetic acid. We find that the 99mTc-labeled **741F8**-1 sFv', specific for the **c-erbB**-2 tumor-associated antigen, is effective in imaging human ovarian carcinoma in a scid mouse tumor xenograft model. This fusion chelate methodology should be applicable to diagnostic imaging with 99mTc and radioimmunotherapy with 186Re or 188Re, and its use could extend beyond the sFv' to other engineered **antibodies**, recombinant proteins, and synthetic peptides.

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9629670 EMBASE No: 95188119

Engineering disulfide-linked single-chain Fv dimers ((sFv)₂) with improved solution and targeting properties: Anti-digoxin 26-10 (sFv)₂ and anti-**c-erbB**-2 **741F8** (sFv)₂ made by protein folding and bonded through C-terminal cysteinyl peptides

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Protein Engineering (United Kingdom) , 1995, 8/3 (301-314) CODEN: PRENE
ISSN: 0269-2139

LANGUAGES: English SUMMARY LANGUAGES: English

Single-chain Fv fusions with C-terminal cysteinyl peptides (sFv) have been engineered using model sFv proteins based upon the 26-10 anti-digoxin IgG and **741F8** anti-**c-erbB**-2 IgG monoclonal **antibodies**. As part of the **741F8** sFv construction process, the PCR-amplified **741F8** V(H) gene was modified in an effort to correct possible primer-induced errors. Genetic replacement of the N-terminal beta-strand sequence of **741F8** V(H) with that from the FR1 of anti-**c-erbB**-2 **520C9** V(H) resulted in a dramatic improvement of sFv folding yields. Folding in urea-glutathione redox buffers produced active sFv' with a protected C-terminal sulfhydryl, presumably as the mixed disulfide with glutathione. Disulfide-bonded (sFv)₂ homodimers were made by disulfide interchange or oxidation after reductive elimination of the blocking group. Both 26-10 (sFv)₂ and **741F8** (sFv)₂ existed as stable dimers that were well behaved in solution, whereas **741F8** sFv and sFv' exhibited considerable self-association. The **741F8** sFv binds to the extracellular domain (ECD) of the **c-erbB**-2 oncogene protein, which is often overexpressed in breast cancer and other adenocarcinomas. The recombinant ECD was prepared to facilitate the analysis of **741F8** binding site properties; the cloned ECD gene, modified to encode a C-terminal Ser-Gly-His6 peptide, was transfected into Chinese hamster ovary cells using a vector that also expressed dihydrofolate reductase to facilitate methotrexate amplification. Optimized cell lines expressed ECD-His6 at high levels in a cell bioreactor; after isolation by immobilized metal affinity chromatography, final ECD yields were as high as 47 mg/l. An animal tumor model complemented physicochemical studies of **741F8** species and indicated increased tumor localization of the targeted **741F8** (sFv)₂ over other monovalent **741F8** species.

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DIALOG(R)File 73:EMBASE
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9326925 EMBASE No: 94276184

Mammalian cell expression of single-chain Fv (sFv) **antibody** proteins and their C-terminal fusions with interleukin-2 and other effector domains
Doral H.; McCartney J.E.; Hudziak R.M.; Tai M.-S.; Laminet A.A.; Houston L.L.; Huston J.S.; Oppermann H.
Creative BioMolecules, Inc., 45 South Street, Hopkinton, MA 01748 USA
BIOTECHNOLOGY (USA), 1994, 12/9 (890-897) CODEN: BTCHD ISSN: 0733-222X

LANGUAGES: English SUMMARY LANGUAGES: English

The production of several single-chain Fv (sFv) **antibody** proteins was examined by three modes of mammalian cell expression. Our primary model was the **741F8** anti-**c-erbB-2** sFv, assembled as either the V(H)-V(L) or V(L)-V(H), and expressed alone, with C-terminal cysteine for dimerization, or as fusion proteins with carboxyl-terminal effector domains, including interleukin-2, the B domain of staphylococcal protein A, the S-peptide of ribonuclease S, or hexa-histidine metal chelate peptide. Constructs were expressed and secreted transiently in 293 cells and stably in CHO or Sp2/O cell lines, the latter yielding up to 10 mg per liter. Single-chain constructs of MOPC 315 myeloma and 26-10 monoclonal **antibodies** were also expressed, as were hybrids comprising unrelated V(H) and V(L) regions. Our results suggest that mammalian expression is a practical and valuable complement to the bacterial expression of single-chain **antibodies**.

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DIALOG(R)File 73:EMBASE
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9198706 EMBASE No: 94146055
gamma-Interferon inhibits Fc receptor II-mediated phagocytosis of tumor cells by human macrophages
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CANCER RES. (USA), 1994, 54/9 (2456-2461) CODEN: CNREA ISSN: 0008-5472

LANGUAGES: English SUMMARY LANGUAGES: English

In vitro, monocyte-derived macrophages (MDM) are capable of efficient **antibody**-mediated phagocytosis of human nucleated tumor cells. These MDM express on their cell surface all three classes of Fc receptors for IgG (FcgammaR). FcgammaR specificity for murine **antibody** isotype allowed us to examine the phagocytic role of FcgammaRII on control and gamma-interferon (IFN-gamma)-primed MDM. Monoclonal **antibody** **520C9** (IgG1) mediates phagocytosis through FcgammaRII. This monoclonal **antibody** is directed against the **HER-2/neu** protooncogene product overexpressed on a variety of adenocarcinomas including the breast carcinoma cell line SK-BR-3. Our results showed that IFN-gamma treatment of differentiated MDM (days 8-12 in culture) inhibited FcgammaRII-mediated phagocytosis in a dose-dependent manner with negative effects noted at doses as low as 0.1 units/ml. The percentage reduction in **antibody**-mediated phagocytosis observed following IFN-gamma priming (40 units/ml for 18 h) ranged from 23-89% of control. The inhibitory effect was evident when exposure to IFN-gamma was transient. FcgammaRII expression was not altered by IFN-gamma treatment. In our model, IFN-gamma did not up-regulate or down-regulate **HER-2/neu** protein expression on our targets or affect the level of CD14 antigen expression on our MDM. Although IFN-gamma is a potent activator of monocytes/macrophages and can enhance certain tumoricidal mechanisms, our data show that **antibody**-dependent phagocytosis through the type II Fc receptor is inhibited by IFN-gamma priming. Nonspecific phagocytosis was not affected.

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DIALOG(R)File 73:EMBASE
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9094524 EMBASE No: 94019199
Immunohistochemical detection of **c-erbB-2** expression by neoplastic human tissue using monospecific and bispecific monoclonal **antibodies**
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INT. J. BIOL. MARKERS (Italy) , 1993, 8/4 (233-239) CODEN: IBMAE

ISSN: 0393-6155

LANGUAGES: English SUMMARY LANGUAGES: English

Selected murine monoclonal **antibodies** (MAB) have been shown to inhibit relevant tumor growth in vitro and in animal models. Recently, bispecific **antibodies** (BsMAB) have been developed which target cytolytic effector cells via one **antibody** binding site and tumor antigen by the other specificity. For example, the BsMAB **2B1** possesses specificity for **c-erbB-2** and FcgammaRIII, the low affinity Fcgamma receptor expressed by polymorphonuclear leukocytes (PMN), macrophages and large granular lymphocytes (LGL). The human homologue of the rat neu oncogene, **c-erbB-2**, has been demonstrated to be amplified in breast, gastrointestinal, lung and ovarian carcinomas. Tumor expression of **c-erbB-2** has been shown to be an important prognostic indicator in breast and ovarian carcinomas. The restricted expression of the **c-erbB-2** protooncogene product in normal human tissues and the wide distribution of **c-erbB-2** expression in such tumors may justify attempts to use an appropriately constructed BsMAB in clinical trials. In this report we have addressed this issue by immunohistochemically evaluating the expression of **c-erbB-2** oncogene product in a variety of malignant tumors utilizing **2B1** and the anti-**c-erbB-2** monovalent parent of **2B1**, **520C9**. Among the studied neoplasms, **c-erbB-2** expression was detected in 49% of primary carcinomas stained with **520C9** and in 39% of those stained with **2B1**. In the group of metastatic tumors, **c-erbB-2** oncoprotein was detected in 52% of cases by **520C9** and in 41% by **2B1**. Our results indicate that immunocytochemistry using bispecific monoclonal **2B1** is a reliable method for the detection of **c-erbB-2** expression, and that this BsMAB detects **c-erbB-2** expression in tumors nearly as well as its anti-**c-erbB-2** monovalent parent **antibody**.

29/3,AB/55 (Item 15 from file: 73)

DIALOG(R)File 73:EMBASE

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8963992 EMBASE No: 93267720

Highly specific in vivo tumor targeting by monovalent and divalent forms of **741F8** anti-**c-erbB-2** single-chain Fv

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CANCER RES. (USA) , 1993, 53/17 (4026-4034) CODEN: CNREA ISSN: 0008-5472

LANGUAGES: English SUMMARY LANGUAGES: English

The in vivo properties of monovalent and divalent single-chain Fv (sFv)-based molecules with the specificity of the anti-**c-erbB-2** monoclonal **antibody** **741F8** were examined in scid mice bearing SK-OV-3 tumor xenografts. **741F8** sFv monomers exhibited rapid, biphasic clearance from blood, while a slightly slower clearance was observed with the divalent **741F8** (sFv)₂ comprising a pair of **741F8** sFv with a C-terminal Gly4Cys joined by a disulfide bond. Following i.v. injection, the **741F8** sFv monomer was selectively retained in **c-erbB-2**-overexpressing SK-OV-3 tumor, with excellent tumor:normal organ ratios uniformly exceeding 10:1 by 24 h. The specificity of this effect was demonstrated by the lack of retention of the anti-digoxin 26-10 sFv monomer, as evaluated by biodistribution studies, gamma camera imaging, and cryomacroautoradiography studies. The specificity index (**741F8** sFv retention/26-10 sFv retention) of **741F8** monomer binding, measured by the percentage of injected dose per g of tissue, was 13.2:1 for tumor, and 0.8 to 2.1 for all tested normal organs, with specificity indices for tumor:organ ratios ranging from 7.0 (kidneys) to 16.7 (intestines). Comparing divalent **741F8** (sFv)₂ with the 26-10 (sFv)₂, similar patterns emerged, with specificity indices for retention in tumor of 16.9 for the Gly4Cys-linked (sFv)₂. These data demonstrate that, following their i.v. administration, both monovalent and divalent forms of **741F8** sFv are specifically retained by SK-OV-3 tumors. This antigen-specific binding, in conjunction with the 26-10 sFv controls, precludes the possibility that passive diffusion and pooling in the tumor interstitium contributes significantly to long-term tumor localization. **741F8**

(sFv)₂ species with peptide spacers exhibited divalent binding and increased retention in tumors as compared with **741F8** sFv monomers. Since the blood retention of the (sFv)₂ is slightly more prolonged than that of the monomer, it was necessary to demonstrate that the increased tumor localization of the peptide-linked (sFv)₂ was due to its divalent nature. The significantly greater localization of the divalent bismalimidohexane-linked **741F8** (sFv)₂ as compared with a monovalent **741F8** Fab fragment of approximately the same size suggests that the increased avidity of the (sFv)₂ is a factor in its improved tumor retention. This is the first report of successful specific in vivo targeting of tumors by divalent forms of sFv molecules. The improved retention of specific divalent (sFv)₂ by tumors may have important consequences for targeted diagnostic or therapeutic strategies.

29/3,AB/56 (Item 16 from file: 73)
DIALOG(R)File 73:EMBASE
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8960827 EMBASE No: 93264554

Binding and cytotoxicity characteristics of the bispecific murine monoclonal **antibody** **2B1**
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J. IMMUNOL. (USA), 1993, 151/5 (2877-2886) CODEN: JOIMA ISSN: 0022-1767

LANGUAGES: English SUMMARY LANGUAGES: English

Bispecific monoclonal **antibodies** (BsmAb) with specificity for tumor Ag and effector cell trigger molecules have been shown to redirect the cytotoxicity of several peripheral blood mononuclear cell populations against relevant tumor. The BsmAb, **2B1**, binds to the extracellular domain of the **c**.*erbB*-2 gene product of the **HER2**/neu proto-oncogene and to CD16. In this report, the binding and cytotoxic characteristics of **2B1** are presented. Maximal saturation binding of **2B1** to PBL and **c**.*erbB*-2 expressing SK-OV-3 cells occurred in the 1 microg/ml concentration range. However, substantial lysis potentiation was observed at 1000-fold lower BsmAb concentrations. Optimal tumor lysis was obtained when the BsmAb, PBL, and target cells were continuously coincubated. When PBL were franked with **2B1**, washed, and added to labeled targets, substantially less lysis was observed. These results suggest that the best way to therapeutically exploit the cytotoxic attributes of **2B1** may be to obtain continuous BsmAb exposure to tumor. Approaches based on franking of this BsmAb to PBL may not be warranted.

29/3,AB/57 (Item 17 from file: 73)
DIALOG(R)File 73:EMBASE
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8715620 EMBASE No: 93019382

A human tumor xenograft model of therapy with a bispecific monoclonal **antibody** targeting **c**.*erbB*-2 and CD16

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CANCER RES. (USA), 1993, 53/1 (94-100) CODEN: CNREA ISSN: 0008-5472

LANGUAGES: English SUMMARY LANGUAGES: English

New strategies are required to clinically exploit the ability of monoclonal **antibodies** to target tumor for lysis by cellular effector mechanisms. In this report we examine the therapeutic effects of **2B1**, a bispecific monoclonal **antibody** with specificity for the extracellular domain of the **c**.*erbB*-2 oncogene product and the human Fcgamma receptor, FcgammaRIII (CD16), describe the characteristics and limitations of this model, and examine the mechanisms underlying the observed responses. The model uses SK-OV-3 human ovarian carcinoma xenografts in scid mice. These cells are susceptible to **2B1**-directed lysis by human peripheral blood lymphocytes or lymphokine-activated killer cells, and maintain **c**.*erbB*-2 expression in vivo. ¹²⁵I-labeled **2B1** selectively accumulates in tumor, with a peak of 10.5% injected dose/g of tumor 24 h following its i.v. injection. However, the selectivity of this

binding is lessened by **2B1** accumulation in the lungs and other normal organs and persistence in the blood. This is caused by **antibody** binding to murine lung, colon, stomach, and skin expressing the epitope recognized by the anti-**c*-erbB*-2** component of **2B1** in tumor-bearing, but not normal mice. In treatment studies using various permutations of **antibody**, human peripheral blood lymphocytes or lymphokine-activated killer cells and interleukin 2, cellular therapy alone had minimal effects on SK-OV-3 xenograft growth, but significantly improved when **2B1** treatment was incorporated. Median survivals increased from 80 plus or minus 3.5 days with no therapy to 131 plus or minus 7.3 days following therapy with 100 microg **2B1**, interleukin 2, and human peripheral blood lymphocytes, with 70% of animals exhibiting no evidence of tumor at day 150. These effects were preserved when the cells were administered in human serum. In contrast, human serum abolished the antitumor effects of **520C9**, which is the parent anti-**c*-erbB*-2** **antibody** of **2B1**. Thus **2B1**-based therapy has therapeutic effects, without obvious toxicity, despite the targeting of this **antibody** to normal murine tissues. Since combinations of **2B1** and interleukin 2 may have antitumor properties, mechanisms other than bispecific monoclonal **antibody**-promoted conjugation of **c*-erbB*-2** antigen-expressing tumor to CD16-expressing effector cells may be involved.

29/3,AB/58 (Item 18 from file: 73)
DIALOG(R)File 73:EMBASE
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8705131 EMBASE No: 93006286

In vitro cytotoxic targeting by human mononuclear cells and bispecific **antibody** **2B1**, recognizing **c*-erbB*-2** protooncogene product and Fcgamma receptor III
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CANCER RES. (USA), 1992, 52/24 (6832-6839) CODEN: CNREA ISSN:
0008-5472

LANGUAGES: English SUMMARY LANGUAGES: English
Bispecific murine monoclonal **antibody** **2B1**, possessing dual specificity for the human **c*-erbB*-2** protooncogene product and human Fcgamma receptor III (CD16) was evaluated for the ability to promote specific lysis of **c*-erbB*-2**-positive tumor cells in vitro. In short-term ⁵¹Cr release assays with human mononuclear cells as effectors and SK-Br-3 human breast cancer cells as targets, neither parental **antibody** of **2B1** mediated significant specific lysis, but bispecific **antibody** was as active as a chemical heteroconjugate, with 5 ng/ml of **2B1** causing half-maximal lysis at an effector/target ratio of 20:1 and 2 ng/ml **2B1** causing half-maximal lysis at an E/T ratio of 40:1. The cytotoxic targeting activity of **2B1** F(ab')₂ fragment was the same as that of whole bispecific **antibody**, and the activity of whole **2B1** was not reduced when assays were performed in 100% autologous human serum, indicating that **2B1** binds effector cells through the CD16-binding site derived from parental **antibody** 3G8 rather than through its Fc portion. Variable inhibition of **2B1**-mediated lysis was observed when autologous polymorphonuclear leukocytes from different donors were added to mononuclear effector cells at a 2:1 ratio; this inhibition was overcome at higher **antibody** concentration. **2B1** bispecific monoclonal **antibody** was also able to mediate targeted cytotoxicity using whole human blood as a source of effector cells or using effector or target cells derived from ovarian cancer patients.

29/3,AB/59 (Item 19 from file: 73)
DIALOG(R)File 73:EMBASE
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8547243 EMBASE No: 92223173

A combination of two immunotoxins exerts synergistic cytotoxic activity against human breast-cancer cell lines
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INT. J. CANCER (USA), 1992, 51/5 (772-779) CODEN: IJCNA ISSN:

0020-7136 ADONIS ORDER NUMBER: 0020713692002968

LANGUAGES: English SUMMARY LANGUAGES: English

In previous studies, combinations of immunotoxins reactive with different cell-surface antigens have exerted additive cytotoxicity against tumor cells in culture. In this report we describe a combination of 2 immunotoxins that produce synergistic cytotoxic activity. Recombinantly derived ricin A chain (RTA) was conjugated with murine monoclonal **antibodies** (MAbs) 317G5, 260F9, 454A12 and **741F8** that bound to cell-surface determinants of 42,55, 180 (transferrin receptor) and 185 kDa (**HER-2** /neu) expressed by the SKBr3 human breast-cancer cell line. When inhibition of clonogenic growth was measured in a limiting dilution assay, the combination of 260F9-RTA and 454A12-RTA produced synergistic cytotoxic activity against SKBr3 and 2 other breast-cancer cell lines. All other combinations produced only additive inhibition of clonogenic growth. Simultaneous binding of 260F9 and 454A12 was not supra-additive, but sub-populations of cells which lacked one or the other antigen could be detected. Kinetic studies of internalization, using **antibodies** conjugated with gold particles, indicated that 454A12 remained within peripheral endosomes for a longer interval in the presence of 260F9. This change in the traffic of the transferrin receptor may contribute to synergy between 260F9-RTA and 454A12-RTA.

29/3,AB/60 (Item 20 from file: 73)

DIALOG(R)File 73:EMBASE

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8217506 EMBASE No: 91246630

Selection of hybrid hybridomas by flow cytometry using a new combination of fluorescent vital stains

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J. IMMUNOL. METHODS (Netherlands) , 1991, 141/2 (165-175) CODEN: JIMMB

ISSN: 0022-1759 ADONIS ORDER NUMBER: 002217599100194R

LANGUAGES: English

A new combination of fluorescent dyes (rhodamine 123 and hydroethidine) was used to internally label hybridoma fusion partners. Murine hybridoma **520C9** (recognizing human **c-erbB** -2) was labeled with hydroethidine. Murine hybridoma 3G8 (recognizing human Fcγ receptor III) was labeled with rhodamine 123, and verapamil was used to block rhodamine efflux via P-glycoprotein. Viability assays showed little cytotoxicity from these dyes at the concentrations used. The labeled cells were fused with polyethylene glycol, sorted for dual fluorescence on an Epics V cell sorter, and cloned. Hybrid hybridomas producing bispecific **antibodies** were selected for ability to promote lysis of SK-Br-3 breast cancer cells by human mononuclear cells. Several positive clones were obtained and shown to have a double content of DNA. Bispecific **antibody** produced by subclone **2B1** was purified by anion exchange chromatography and shown to bind both tumor cells and Fcγ₃ bearing cells. Using two parameter flow cytometric analysis, we were able to measure a 'bridging' effect of this bispecific **antibody**, which caused formation of complexes between PMNs and SK-Br-3 cells. Either parental **antibody** could compete with bispecific **antibody** to block such complexing. This fusion method provides several advantages over other techniques presently used (speed, convenience, low toxicity and automatic exclusion of dead cells) and can be applied to produce other hybrid hybridomas.

29/3,AB/61 (Item 21 from file: 73)

DIALOG(R)File 73:EMBASE

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8207104 EMBASE No: 91236534

Identity of BCA200 and **c-erbB** -2 indicated by reactivity of monoclonal **antibodies** with recombinant **c-erbB** -2

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MOL. IMMUNOL. (United Kingdom) , 1991, 28/8 (915-917) CODEN: IMCHA

ISSN: 0161-5890 ADONIS ORDER NUMBER: 016158909100142I

LANGUAGES: English

BCA200 has been described as a 200,000 Mr monomeric cell surface glycoprotein associated with human breast cancer. Since the physical properties and cellular distribution of BCA200 resemble those of **c-erbB-2**, **antibodies** to BCA200 were tested for the ability to bind a recombinant protein containing the **c-erbB-2** extracellular domain (erbB-2 ECD). Three **antibodies** to distinct epitopes of BCA200 reacted with erbB-2 ECD but not with a control protein expressed in a similar baculovirus lysate. Control myeloma proteins and **antibodies** to four other antigens did not react with erbB-2 ECD. A protein with the expected molecular weight for erbB-2 ECD was also immunoprecipitated by anti-BCA200 **antibody** **520C9**. We conclude that BCA200 is another synonym for **c-erbB-2**.

FILE 'USPAT' ENTERED AT 09:44:37 ON 11 OCT 96

* WELCOME TO THE *
* U.S. PATENT TEXT FILE *

L1 437 NEU OR CERBB2 OR C ERB B# OR C ERBB 2 OR HER2 OR HER 2 OR H
L2 52 BISPECIFIC ANTIBOD?
(BISPECIFIC(W)ANTIBOD?)
L3 64 CD16
L4 3 L2 AND L3
L5 0 L1 AND L4
L6 0 L1 AND L2
L6 0 L1 AND L2
L7 10 "RING, DAVID B"/IN

=> d 14 cit ab parn 1-

1. 5,556,620, Sep. 17, 1996, Use of recombinant colony stimulating factor-1 to enhance wound healing; Peter Ralph, et al., 424/85.1, 278.1; 514/8, 12, 885, 887 [IMAGE AVAILABLE]

US PAT NO: 5,556,620 [IMAGE AVAILABLE] L4: 1 of 3

ABSTRACT:

A colony stimulating factor, CSF-1, is a lymphokine useful in treating or preventing bacterial, viral or fungal infections, neoplasms, leukopenia, wounds, and in overcoming the immunosuppression induced by chemotherapy or resulting from other causes. CSF-1 is obtained in usable amounts by recombinant methods, including cloning and expression of the murine and human DNA sequences encoding this protein.

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation of U.S. patent application Ser. No. 08/024,094, filed 26 Feb. 1993, now abandoned, which is a continuation of U.S. patent application Ser. No. 07/505,256, filed 5 Apr. 1990, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07/243,253, filed 14 Sep. 1988, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07/099,872, filed 22 Sep. 1987, now U.S. Pat. No. 5,104,650, which is a continuation-in-part of U.S. patent application Ser. No. 06/876,819, filed 20 Jun. 1986, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 06/821,068, filed 21 Jan. 1986, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 06/756,814, filed 18 Jul. 1985, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 06/744,924, filed 14 Jun. 1985, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 06/728,834, filed 30 Apr. 1985, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 06/698,359, filed 5 Feb. 1985, now abandoned.

2. 5,547,668, Aug. 20, 1996, Conjugates of folate anti-effector cell antibodies; David M. Kranz, et al., 424/181.1, 154.1, 173.1, 178.1; 435/70.21, 172.2, 240.27; 530/388.75, 389.6, 391.1, 391.7 [IMAGE AVAILABLE]

US PAT NO: 5,547,668 [IMAGE AVAILABLE] L4: 2 of 3

ABSTRACT:

The present invention provides a process of targeting folate-receptor-positive tumor cells for lysis by binding a conjugate of folate and an anti-T-cell-receptor antibody or an anti-Fc receptor antibody to those cells. A process of lysing folate-receptor-positive tumor cells comprising exposing the cells to a folate/anti-T-cell-receptor antibody in the presence of a population of T-cells is also provided. A process of lysing folate-receptor-positive tumor cells comprising exposing the cells to a folate/anti-Fc receptor antibody in the presence of a population of natural killer cells, monocytes, or

macrophages is also provided. Still further, the present invention provides a conjugate of folate to an anti-T-cell-receptor antibody or an anti-Fc receptor antibody.

3. 5,422,105, Jun. 6, 1995, Use of recombinant colony stimulating factor 1; Peter Ralph, et al., 424/85.1; 252/301.27; 424/85.2, 404; 435/254.1; 514/8, 12; 530/350, 351 [IMAGE AVAILABLE]

US PAT NO: 5,422,105 [IMAGE AVAILABLE] L4: 3 of 3

ABSTRACT:

A colony stimulating factor, CSF-1, is a lymphokine useful in treating or preventing bacterial, viral or fungal infections, neoplasms, leukopenia, wounds, and in overcoming the immunosuppression induced by chemotherapy or resulting from other causes. CSF-1 is obtained in usable amounts by recombinant methods, including cloning and expression of the murine and human DNA sequences encoding this protein.

PARENT-CASE:

This application is a continuation of application Ser. No. 08/017,963, filed Feb. 12, 1993, now abandoned, which is a continuation of application Ser. No. 07/572,149, filed Aug. 23, 1990, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 505,256, filed Apr. 5, 1990, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 243,253, filed Sep. 14, 1988, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 99,872, filed Sep. 22, 1987, which is a continuation-in-part of U.S. patent application Ser. No. 876,819, filed Jun. 20, 1986, now abandoned, (which was refiled as U.S. patent application Ser. No. 157,094, filed Feb. 2, 1988 and issued as U.S. Pat. No. 4,847,201), which is a continuation-in-part of U.S. patent application Ser. No. 821,068, filed Jan. 21, 1986, (now abandoned), which is a continuation-in-part of U.S. patent application Ser. No. 756,814, filed Jul. 18, 1985, (now abandoned), which is a continuation-in-part of U.S. patent application Ser. No. 744,924, filed Jun. 14, 1985, (now abandoned), which is a continuation-in-part of U.S. patent application Ser. No. 728,834, filed Apr. 30, 1985, (now abandoned), which is a continuation-in-part of U.S. Ser. No. 698,359, filed Feb. 5, 1985, (now abandoned). All of these prior applications are hereby incorporated by reference in their entirety.

=> d 17 cit ab parn 1-

1. 5,534,254, Jul. 9, 1996, Biosynthetic binding proteins for immuno-targeting; James S. Huston, et al., 424/135.1, 143.1, 155.1, 178.1; 530/387.3, 388.22, 388.8, 389.7, 391.1, 391.7, 391.9 [IMAGE AVAILABLE]

US PAT NO: 5,534,254 [IMAGE AVAILABLE] L7: 1 of 10

ABSTRACT:

Disclosed is a formulation for targeting an epitope on an antigen expressed in a mammal. The formulation comprises a pharmaceutically acceptable carrier together with a dimeric biosynthetic construct for binding at least one preselected antigen. The biosynthetic construct contains two polypeptide chains, each of which define single-chain Fv (sFv) binding proteins and have C-terminal tails that facilitate the crosslinking of two sFv polypeptides. The resulting dimeric constructs have a conformation permitting binding of a said preselected antigen by the binding site of each said polypeptide chain when administered to said mammal. The formulation has particular utility in in vivo imaging and drug targeting experiments.

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of co-pending application Ser. No. 831,967 filed Feb. 6, 1992, incorporated herein by reference.

2. 5,169,774, Dec. 8, 1992, Monoclonal anti-human breast cancer

antibodies; Arthur E. Frankel, et al., 530/388.85; 435/7.23, 172.2, 240.27; 436/64, 548, 813; 530/808, 864; 935/106, 110 [IMAGE AVAILABLE]

US PAT NO: 5,169,774 [IMAGE AVAILABLE] L7: 2 of 10

ABSTRACT:

Murine monoclonal antibodies, or fragments thereof, that bind selectively to human breast cancer cells, are IgGs or IgMs, and when conjugated to ricin A chain, exhibit a TCID 50% against at least one of MCF-7, CAMA-1, SKBR-3, or BT-20 cells of less than about 10 nM. Methods for diagnosing, monitoring, and treating human breast cancer with the antibodies or immunotoxins made therefrom are described.

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 842,476, filed Mar. 21, 1986, which is a continuation-in-part of U.S. Ser. No. 690,750, now U.S. Pat. No. 4,753,894, filed Jan. 11, 1985, which is a continuation-in-part of U.S. Ser. No. 577,976, filed Feb. 8, 1984, now abandoned.

3. 4,958,009, Sep. 18, 1990, Anti-human ovarian cancer immunotoxins and methods of use thereof; Michael J. Bjorn, et al., 424/183.1, 155.1, 156.1, 804, 807; 514/885; 530/388.8, 388.85, 391.7, 808, 864 [IMAGE AVAILABLE]

US PAT NO: 4,958,009 [IMAGE AVAILABLE] L7: 3 of 10

ABSTRACT:

Immunotoxins comprising a cytotoxic moiety and monoclonal antibodies which bind to human ovarian cancer tissue having at least one of the following capabilities are claimed: cytotoxic ID.sub.50 of 10 nM or less against human ovarian cancer cells, retardation of human ovarian cancer tumor growth in mammals or extension of survival of a mammal carrying a human ovarian cancer tumor. Antigens to which the monoclonal antibody of the immunotoxin bind are identified and characterize the immunotoxins. Methods of killing human ovarian cancer cells, retarding the growth of human ovarian cancer tumors in mammals or extending the survival of mammals carrying human ovarian cancer tumors are claimed.

PARENT-CASE:

This is a continuation of application Ser. No. 806,320, filed Dec. 6, 1985 now abandoned.

4. 4,956,453, Sep. 11, 1990, Anti-human ovarian cancer immunotoxins and methods of use thereof; Michael J. Bjorn, et al., 424/183.1, 155.1, 156.1, 804, 807; 514/885; 530/388.22, 388.8, 388.85, 391.7, 808, 861, 864, 866 [IMAGE AVAILABLE]

US PAT NO: 4,956,453 [IMAGE AVAILABLE] L7: 4 of 10

ABSTRACT:

Immunotoxins comprising a cytotoxic moiety and an antigen binding portion selected from the group consisting of Fab, Fab' and F(ab').sub.2 fragments of a monoclonal antibody, which binds to human ovarian cancer tissue, having one of the following capabilities are claimed: cytotoxic ID.sub.50 of about 10 nM or less against human ovarian cancer cells, retardation of human ovarian cancer tumor growth in mammals, or extension of survival of a mammal carrying a human ovarian cancer tumor. Antigens or epitopes to which the monoclonal antibodies bind are identified and characterize the immunotoxins. In a preferred embodiment an immunotoxin comprising at least an antigen binding portion of a monoclonal antibody, which binds to human transferrin receptor, but does not block binding of transferrin to the receptor, is described and claimed. Immunotoxin comprising the F(ab').sub.2 region of the antitransferrin monoclonal antibody are also claimed. Methods of killing human ovarian cancer cells, retarding the growth of human ovarian cancer tumors in mammals and extending the survival of mammals carrying human ovarian cancer tumors are claimed.

PARENT-CASE:

This is a continuation of application Ser. No. 806,256, filed Dec. 6, 1985.

5. 4,938,948, Jul. 3, 1990, Method for imaging breast tumors using labeled monoclonal anti-human breast cancer antibodies; **David B. Ring**, et al., 424/1.53, 9.34, 9.43, 9.6, 155.1, 156.1, 179.1, 804, 807; 435/172.2, 948; 530/388.2, 388.8, 391.3, 391.5, 402, 808, 861; 935/102, 107 [IMAGE AVAILABLE]

US PAT NO: 4,938,948 [IMAGE AVAILABLE] L7: 5 of 10

ABSTRACT:

Hybridomas producing monoclonal antibodies suitable for imaging and diagnosis of human breast tumors and such monoclonal antibodies are claimed. The monoclonals are characterized by breast tumor binding range, breast cancer cell line range, and selectivity. Immunoinaging agents comprising the monoclonal antibody and a detectable label, either directly or indirectly conjugated to the antibody are claimed. Methods for imaging breast tumors using the immunoinaging agents are described and claimed.

PARENT-CASE:

This patent application is a continuation-in-part application of copending U.S. Application Ser. No. 785,076 filed Oct. 7, 1985, now abandoned.

6. 4,803,169, Feb. 7, 1989, Assay for human breast cancer; Peter S. Linsley, et al., 435/7.23, 7.9, 7.95, 240.27; 436/501, 518, 536, 548, 813; 530/388.85 [IMAGE AVAILABLE]

US PAT NO: 4,803,169 [IMAGE AVAILABLE] L7: 6 of 10

ABSTRACT:

Methods are disclosed for detecting, staging and monitoring breast cancer. The methods involve determining the amount of certain antigens, designated W1 and W9, in serum via quantitative immunoassays using anti-W1 or anti-W9 monoclonal antibodies. The amount detected is compared with amounts of the antigen found in normal sera, sera from breast cancer patients of predetermined stage, or other samples of the patient's own serum, depending upon the purpose of the assay.

7. 4,753,894, Jun. 28, 1988, Monoclonal anti-human breast cancer antibodies; Arthur E. Frankel, et al., 435/7.23; 424/155.1, 156.1, 183.1, 804; 435/7.95, 240.27, 948; 530/388.85, 391.3, 391.7, 402, 808, 861, 864; 935/104, 107 [IMAGE AVAILABLE]

US PAT NO: 4,753,894 [IMAGE AVAILABLE] L7: 7 of 10

ABSTRACT:

Murine monoclonal antibodies are prepared and characterized which bind selectively to human breast cancer cells, are IgGs or IgMs, and when conjugated to ricin A chain, exhibit a TCID 50% against at least one of MCF-7, CAMA-1, SKBR-3, or BT-20 cells of less than about 10 nM. Methods for diagnosing, monitoring, and treating human breast cancer with the antibodies or immunotoxins made therefrom are described.

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of copending U.S. application Ser. No. 577,976 filed Feb. 8, 1984.

8. 4,727,037, Feb. 23, 1988, Assay kit and method for the determination of antibody class and subclass; **David B. Ring**, 436/548; 435/7.92, 240.26, 805, 810, 970; 436/513, 530, 808, 809, 810; 935/103, 110 [IMAGE AVAILABLE]

US PAT NO: 4,727,037 [IMAGE AVAILABLE]

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ABSTRACT:

A method of rapid determination of the isotype class for a panel of monoclonal antibodies is described. The assay comprises adsorbing on a solid support medium antibodies directed to specific immunoglobulin heavy and light chains. Once such isotype-specific antibodies are bound to the nitrocellulose paper, the treated strips can be incubated with the monoclonal antibody of interest. Upon formation of a complex between the specific iso-type antisera and the monoclonal antibody, the complex is visualized by reaction with a chromogenic substance. In the preferred embodiment of the invention, the treated nitrocellulose strips are stored in kit form. Using these prepared strips, the isotyping assay can be performed in less than two hours with a minimum of technical manipulation and expenditure of reagents.

9. 4,569,915, Feb. 11, 1986, P. obtusus strain; **David B. Ring**, 435/254.1, 105, 190, 911 [IMAGE AVAILABLE]

US PAT NO: 4,569,915 [IMAGE AVAILABLE]

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ABSTRACT:

A P. obtusus strain AU124 having the identifying characteristics of NRRL No. 15595, the strain produces high levels of pyranose-2-oxidase.

10. 4,568,645, Feb. 4, 1986, Fungal pyranose-2-oxidase preparations; Kirston E. Koths, et al., 435/190, 911 [IMAGE AVAILABLE]

US PAT NO: 4,568,645 [IMAGE AVAILABLE]

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ABSTRACT:

A substantially pure pyranose-2-oxidase preparation obtained from C. versicolor or L. betulinus. The preparation is substantially free of glucosone-utilizing enzyme contaminants having measurable activity at a pH between about 4.4 and 7.0.